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A STUDY TO MONITOR THE IMPACTS OF THE 1986 HEMLOCK LOOPER INSECTICIDE SPRAY PROGRAM ON SONGBIRDS

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THE EFFECTS OF FENITROTHION ON FOREST SONGBIRD BRAIN CHOLINESTERASE ACTIVITY

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EXECUTIVE SUMMARY

The Newfoundland and Labrador Department of Forest Resources and Lands (DFRL) conducted an aerial spray program to control an outbreak of the eastern hemlock looper (Lambdina fiscellaria fiscellaria) in insular Newfoundland during the summer of 1986. The insecticide used in the spray application was fenitrothion.

The DFRL was directed by the Newfoundland Department of Environment (NDOE) to undertake a monitoring study to assess the impact of fenitrothion on forest songbird populations.

The fenitrothion monitoring study area was located in, and adjacent to the DFRL spray block #107 near Harpoon Brook, approximately 20 km south of Millertown, Newfoundland. The study area is within the Central Newfoundland Ecoregion (Damman 1983). Much of the study area was cut over in the mid to late 1960's. Now the area is primarily composed of immature black spruce (Picea mariana) and balsam fir (Abies balsamea) stands. There are some remaining areas of mature black spruce. Some areas were not sufficiently restocked with trees after cutting operations were completed, according to Forest Inventory Maps.

The methodology chosen for the monitoring study was as follows:

- forest songbirds were collected using .410 calibre shotguns
- songbirds were collected from unsprayed areas for comparison

to songbirds collected after each of two fenitrothion spray applications

- songbird brains were removed and stored for later analysis for cholinesterase activity
- results were analyzed and compared with the literature.

Field Results

The study team was in the field from July 14 to August 5. The lack of mature forest in the study area made sampling of upper canopy forest songbirds difficult. In addition, the late spraying dates, July 27 and August 2 and 3, caused difficulties for the study team in locating songbirds. The breeding season for some species was nearly completed and was completed for others. Thus birds were not singing and as a result were inconspicuous.

In spite of these restrictions during the field study a total of 113 songbirds were collected from which analysis could be made. Brain cholinesterase (ChE) activity was analyzed for statistical and biological significance. Comparisons between sprayed and unsprayed samples of the same species of songbird were made.

All sample groups, (e.g. unsprayed, collected after first spray and collected after second spray for each species) with more than 2 samples were tested for normality. All such groups proved to

have a normal distribution. There was no statistically significant difference in the ChE activity amongst any of the of the songbird species collected from unsprayed areas. After the first spray application there was a statistically significant reduction of brain ChE activity for all songbird species compared to the unsprayed sample of the same species. After the second spray application there was a statistically significant reduction in brain ChE activity in white-throated sparrows compared to unsprayed white-throated sparrows. Although there was a reduction in brain ChE activity observed in yellow-rumped warblers, the reduction wasn't statistically significant.

In this study, 39 (70.9%) of the songbirds collected after the fenitrothion spray applications had brain ChE activity indicative of being exposed to a ChE inhibiting agent according to the criterion of Zinkl et al. (1980). A total of 21 (38.1%) of the songbirds collected after the fenitrothion spraying had potentially life threatening brain ChE inhibition according to the criterion of Zinkl et al. (1980). In contrast, 1 (1.8%) brain sample from the unsprayed songbirds had a brain ChE activity indicating exposure to a ChE inhibiting agent.

Conclusions

Based on the results of this study there is reason to be concerned about the effects of fenitrothion spraying operations

at 210 g AI/ha on forest songbirds in Newfoundland. Evidence from this study suggests that potentially life threatening exposure to fenitrothion of some songbirds sampled (38.1%) had occurred according to the criterion of Zinkl et al. (1980).

Although this is a monitoring study the results from fenitrothion spraying events are largely descriptive of the individual spray event and evidence suggests that caution should be taken before making broad predictions of all fenitrothion spraying events from one monitored event. Variable weather conditions can prevent a spray operation from being a near perfect event. The 1985 and 1986 songbird monitoring studies conducted by DFRL are a case in point. Both studies monitored the effects on songbird brain ChE after an operational spray application of 210 g AI/ha fenitrothion. Strong and Wells (1985) determined that 7% of songbirds sampled were exposed to a potentially lethal dose of a cholinesterase inhibiting agent while in this study 38.1% of the songbirds collected were exposed to a potentially lethal dose of ChE inhibitor. The results from the Codroy Pond overspray incident (Addendum) show approximately 42.8% of the songbirds collected were exposed to a potentially lethal dose of a ChE inhibitor. Although the results of the Codroy overspray incident are quite similar to the results observed in the 1986 study, fenitrothion was accidentally applied at a rate of 350 g AI/ha (over 1.6 x the normal application rate). These results reveal that it may not be possible to use the results of a single spray event monitoring study to reliably predict the impact of other

spray events on forest songbirds. However, songbird monitoring studies of fenitrothion spray events are useful for describing the adverse effects of the specific spray event.

However, this study does suggest there is reason to be concerned about the effects on songbirds during fenitrothion spray applications at 210 g AI/ha. The adverse effects of fenitrothion on forest songbirds are well documented (see Section 1.0) and forest managers should continue to monitor the potential adverse impact of fenitrothion on forest songbirds as part of their forest management programs.

Recommendations

In view of the measured adverse effect of fenitrothion applied at 210 g AI/ha on forest songbird brain ChE activity in this study, the DFRL should continue their research and development of alternative forest insect control sprays, formulations and application rates.

It is recommended that future monitoring efforts be initiated as early in the spray program as possible. The contract for this monitoring study was assigned on 11 July 1986 with the first spray delivered 27 July 1986. Spraying operations had been underway since early July on the west coast of insular Newfoundland. By the end of July in central Newfoundland some

species of forest songbirds (magnolia warbler in particular) had already started to migrate. The collection of birds during migration could result in underestimating the effect of ChE inhibitors.

It is recommended, should brain ChE inhibiting agents be used in future forest spray programs, that continued and more extensive monitoring programs be implemented. More operational spray and control areas should be monitored for the effects of insecticide spray on forest songbird populations. Efforts should be made to continue the brain ChE monitoring program to provide annually consistent collection methods to enable monitors to make valid comparisons and compilations of data.

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1.0 INTRODUCTION

The Newfoundland and Labrador Department of Forest Resources and Lands (DFRL) used fenitrothion during the summers of 1985 and 1986 to control predicted outbreaks of eastern hemlock looper (Lambdina fiscellaria fiscellaria) in insular Newfoundland. DFRL was directed by the Newfoundland Department of Environment in 1986 to undertake a monitoring study on the impact of the spray application on songbirds.

Fudge, Lane & Associates Limited was contracted to conduct the 1986 songbird moritoring study to determine the effect of fenitrothion on songbirds.

The widespread and long-term use of insecticides for the control of the eastern spruce budworm (Choristoneura fumiferana) in eastern Canada has been reviewed by Mineau and Peakall (1984). In eastern Canada, eastern spruce budworm control operations have resulted in organochlorines, organophosphates and carbamates being broadcast over balsam fir (Abies balsamea) and spruce (Picea spp.) forests (Mineau and Peakall 1984, Peakall and Bart 1983).

Fenitrothion (0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothioate) has been the major insecticide used to control the eastern spruce budworm since 1968 (Busby et al. 1983). Fenitrothion, an organophosphate, is a

1968 (Busby et al. 1983). Fenitrothion, an organophosphate, is a cholinesterase (ChE) inhibitor (Silver 1975).

ChE inhibitors have been used increasingly as insecticides since the initiation of the bans or restrictions on chlorinated hydrocarbons (Zinkl et al, 1979). ChE inhibitors block synaptic transmissions in the cholinergic parts of the nervous system by binding to the active site of acetylcholinesterase, which normally hydrolyses the neurotransmitter acetylcholine to choline and acetic acid. The ChE inhibitors thus permit excessive acetycholine accumulation at synapses and disrupt or block nerve impulse transfer (Corbett 1974). Acetylcholinesterase inhibition probably causes death in air-breathing vertebrates by blocking neurotransmissions in the respiratory centre of the brain or at myoneural junctions of respiratory muscles (O'Brien 1967, Corbett 1974). Unintentional mortality of songbirds has been reported from fenitrothion use in forest insect control programs (Grue et al. 1983).

Fenitrothion is applied aerially to the forest canopy thereby exposing many non-target organisms, including songbirds to the toxin (Busby et al. 1983).

Avian response to insecticide spraying has traditionally been monitored by comparing pre- and post-spray singing activity of territorial males, a decrease in singing

activity being assumed to indicate a detrimental impact (Pearce et al. 1976, 1979; Busby et al. 1983). Singing bird surveys are confounded by non-uniform spray application, replacement of birds killed (Stewart and Aldrich 1951, Hensley and Cope 1951), adverse weather conditions and bird migration (Pearce et al. 1976, Peakall and Bart 1983). Other signs of ChE inhibitor poisoning in birds include myosis, salivation, lacrimation (muscurinic effects), muscle twitching, dyspnoea, paralysis and clonic convulsions (nicotinic effects) (Zinkl et al. 1977). In addition, the fate of most birds in areas of reduced singing activity remain unknown (Busby et al. 1983).

Since bird ChE activity is easily measured, its measurement can be used to determine if an animal has been poisoned with organophosphate or carbamate insecticides (Stickel 1974). Brain ChE measures an adverse physiological response (Busby et al. 1983). However, the ChE activity of birds suspected to have been sprayed with ChE inhibitors must be compared with that of unsprayed birds of the same species because of the variation in ChE activity between species (Stickel 1974).

Brain ChE determination is becoming widely accepted as a method of assessing exposure of birds to ChE inhibiting insecticides (White et al. 1979; Zinkl et al. 1979; Busby et al. 1981; Zinkl et al. 1981; Busby et al. 1983).

1.1 Objectives

The objective of this study was to assess the effects on forest songbirds of the DFRL fenitrothion spray application to control the eastern hemlock looper in insular Newfoundland. Fudge, Lane and Associates Limited used brain ChE determination to achieve this objective.

1.2 Study Area

The study area was located about 20 km south of Millertown, Newfoundland, within the Central Newfoundland Ecoregion (Damman 1983). The area is characterized by balsam fir (Abies balsamea) and black spruce (Picea mariana) forests. Stands of trembling aspen (Populus tremuloides) and white birch (Betula papyrifera) are common in the study area. Scrub areas of aspen and alder (Alnus rugosa) and regenerating cut-over areas also occur in the study area. Dwarf shrub heath dominated by Kalmia angustifolia and small areas of Sphagnum spp. bog can occur locally.

Much of the study area was cut over in the mid to late 1960's. The regenerated areas are composed of immature (0-20 years) black spruce and balsam fir under 3.5 m in

height, some mature black spruce (41-60 and 61-80 years) 9.6-12.5 m high and some areas were not sufficiently restocked (Forest Inventory Maps DFRL). The location of the study area is shown in Figure 1.1.

1.3 Materials and Methods

Operational spray block #107 was used for the 1986 songbird monitoring study.

Bird species to be collected were chosen prior to the field work so that all strata of the target forest were hopefully represented, presence in both control and spray blocks being a criterion. The blackpoll warbler (BP) (Dendroica striata) represented the upper canopy. The yellow-rumped warbler (YRW) (Dendroica coronata) represented the mid to upper canopy and the magnolia warbler (MAG) (Dendroica magnolia) typically forages in the mid to lower canopy. White-throated sparrows (WTS) (Zonotrichia albicollis) are low canopy to ground foragers.

The field crew was established in the study area on 14 July 1986. Collecting of birds for the control sample commenced after a reconnaissance of the study area. After realizing a paucity of warblers in the study area

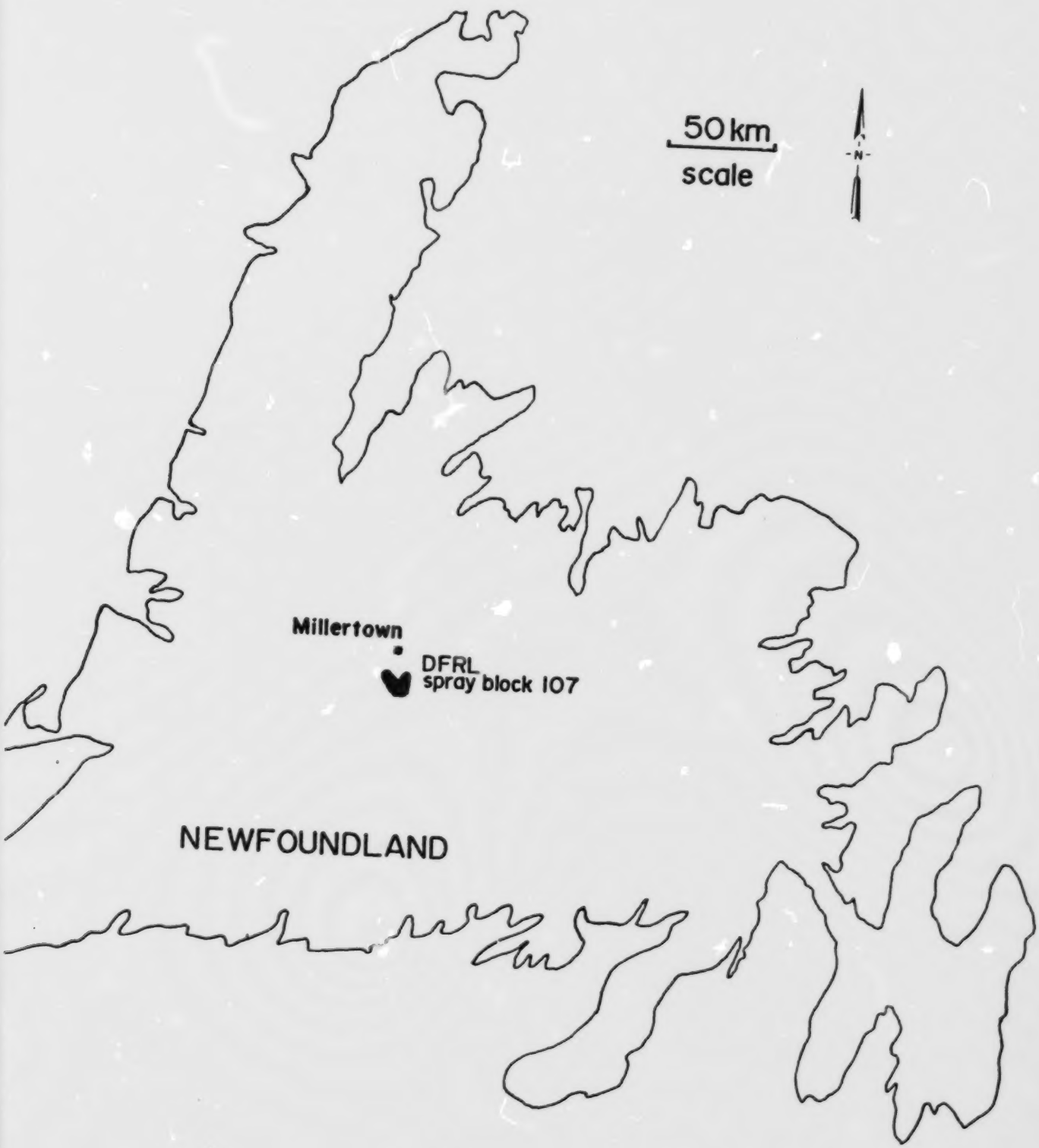

Figure 1.1 Location of study area.

the field crew requested permission to collect additional species of warblers; black-throated green warbler (BTG) (Dendroica virens), northern water-thrush (NW) (Seiurus noveboracensis), Wilson's warbler (WW) (Wilsonia pusilla) and black and white warbler (BW) (Mniotilta varta).

A portion (10 swaths) of block #107 was sprayed to enable the monitoring study to proceed. Spraying was conducted using one DC-4 aircraft equipped with boom and nozzle spray apparatus.

Technical details of spray application and mixture are provided in Table 1.1.

The first fenitrothion spray application was at 21:00 on 27 July 1986. The second spray application was partially completed from 21:15 to 22:00 on 2 August 1986 and completed from 07:30 to 08:00 on 3 August 1986. Most control birds were collected before the first spray application although 10 control samples were collected after the second spray application. No control samples were collected after the second spray application. All birds were collected by shooting (.410 shotgun with #7½ and #6½ shot). Efforts were made to collect non-singing individuals, however many birds were located by sound. Areas close to the spray

Table 1.1: Technical details of spray application and formulation for 1986 hemlock looper control program.

Spray Application

Aircraft:	DC-4
Aircraft height:	61 m
Aircraft speed:	305 km/h
Spray apparatus:	Boom and nozzle with #8010 spray tip

Spray Mixture

Insecticide:	Fenitrothion (Folithion®)
Mixture by Volume:	11% fenitrothion 40% Cyclosol 63 49% ID 585 (Insecticide Diluent)

Application Rate

210 g active ingredient per 1.5 L per ha in 2 applications.

Application Date

1st. spray 27 July 1986 (evening)
2nd. spray-partial 2 August 1986 (evening)
-partial 3 August 1986 (morning)

block boundaries were avoided to reduce the chance of collecting unexposed birds that flew into the sprayed area after spraying had ceased.

After shooting, specimens were labelled and taken to a pre-arranged area where a cryogenic container (liquid nitrogen) was located. Brains were then removed from the specimens, placed in serum vials and stored in the liquid nitrogen until time of assays. Generally, specimens were processed and brains were stored in the liquid nitrogen within 1 h of collecting and never longer than 2 h. Bird brains were assigned a code of 0 for having no shot damage, 1 for having little shot damage and 2 for having a considerable portion of the brain missing. Brains which had received heavy shot damage were not used in the analyses because different areas of a bird brain are reported to have different ChE activities (Knittle and Tucker 1974, from Zinkl, Henny and DeWeese 1977). Brain ChE assays were conducted by the Canadian Wildlife Service, Fredericton, New Brunswick and followed the colorimetric techniques of Ellman et al. (1961) as adapted by Hill and Fleming (1982).

Prior to assaying, all samples were randomized to prevent possible bias from processing sequential groups of specimens under laboratory conditions which can vary

slightly from day to day. Brain analysis was completed on 4 September 1986.

2.0 RESULTS

It was not possible for the field crew to partition songbird collecting on the basis of upper canopy species, mid-canopy species and ground vegetation species. Most of the study area was regeneration vegetation and the tallest stands of trees were only 8-11 m in height. Results were therefore analyzed for changes in ChE activity within species groups before and after spraying. No dead songbirds were found and no behavioral abnormalities were observed in the field.

The results of brain ChE laboratory assays were analyzed for statistical and biological significance.

Spray block #107 and songbird collection areas shown in Figure 2.1.

2.1 Statistical Significance

All sample groups (WTS unsprayed, WTS spray 1, WTS spray 2, YRW unsprayed, YRW spray 1, YRW spray 2,

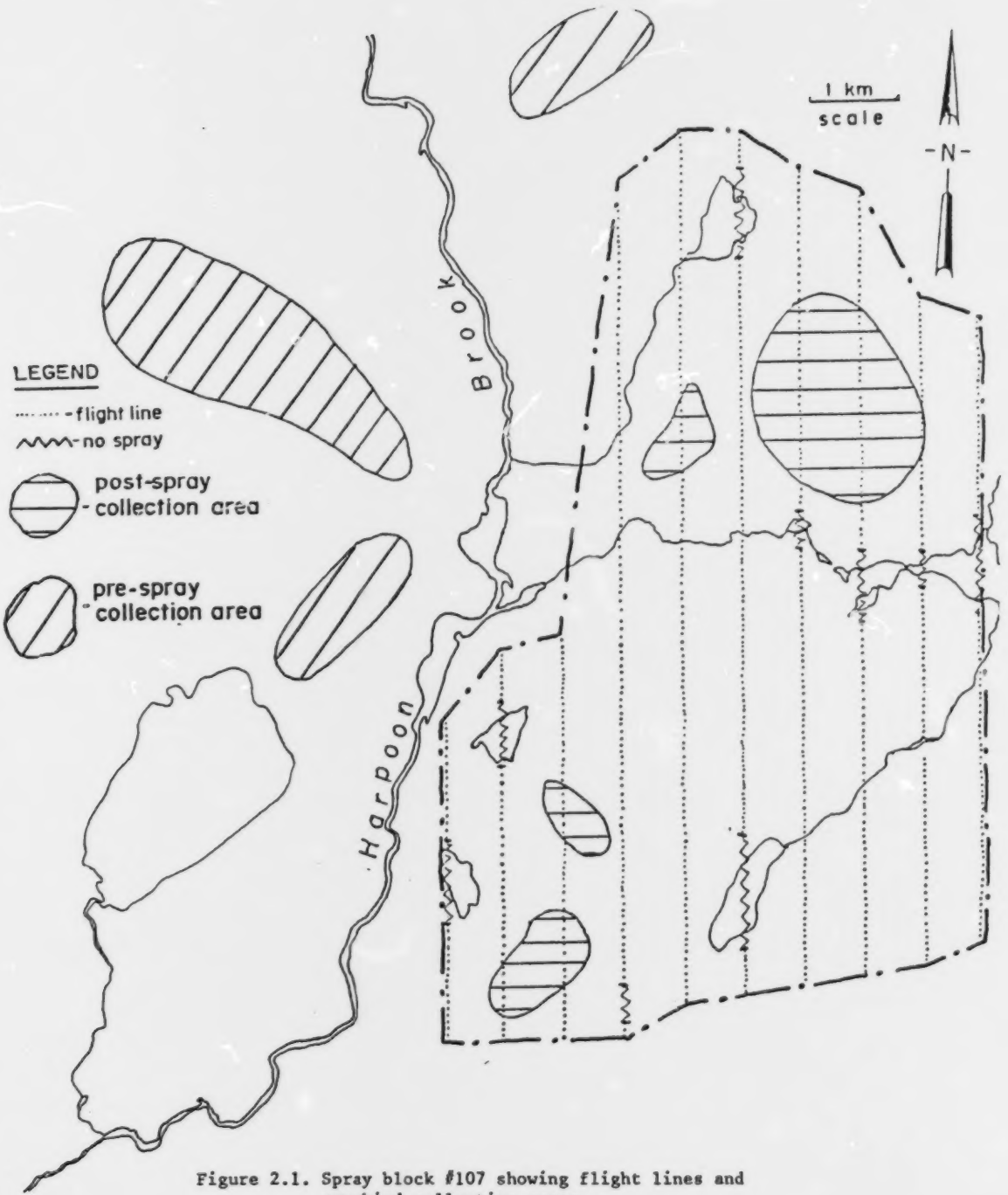

Figure 2.1. Spray block #107 showing flight lines and songbird collection areas.

BP unsprayed, MAG unsprayed, MW spray 1) were tested for a normal distribution. No BW were collected after fenitrothion applications. Only one MW was collected after the second fenitrothion application. All sample groups had a normal distribution which allowed further testing for differences between their mean values for ChE activity. Sample groups with 1 or 2 samples were not tested for normality and therefore were not used in comparisons of means. There was no significant difference in the mean ChE activity among any of the bird species groups collected from the unsprayed areas (Table 2.1).

After the first spray application there was a significant reduction in brain ChE activity for all species groups compared to the unsprayed sample of the same species (Table 2.2).

There was a significant reduction in the mean brain ChE activity in WTS spray 2 compared to WTS unsprayed. No significant reduction was found in YRW spray 2 compared to YRW unsprayed although the mean brain ChE activity was lower (Table 2.2).

When the brain specimens were collected in the field they were coded with either 0, 1 or 2 depending on

TABLE 2.1: Brain ChE activities (μ moles/min/g) of songbirds collected from unsprayed forests

Species	Sample Size	Mean $\bar{X}$ (SD)*	$\bar{X}$ - 2 SD (#/% Sample)	$\bar{X}$ - 0.2 X (#/% Sample)	$\bar{X}$ - 0.5 $\bar{X}$ (#/% Sample)
White-throated Sparrow	23	29.39 (3.64)	22.11** (1/4.3%)	23.51 (2/8.7%)	14.70 (0/0.0%)
Yellow-rumped Warbler	15	28.56 (4.87)	18.82** (0/0.0%)	22.85 (2/13.3%)	14.28 (0/0.0%)
Blackpoll Warbler	11	33.67 (7.23)	19.21** (0/0.0%)	26.94 (1/9.1%)	16.84 (0/0.0%)
Magnolia Warbler	5	31.19 (3.40)	24.39** (0/0.0%)	24.95 (0/0.0%)	15.60 (0/0.0%)
Black and White Warbler	2	25.99 (2.35)	21.28 (0/0.0%)	20.97** (0/0.0%)	13.00 (0/0.0%)
Wilson's Warbler	1	32.65 (-)	-	26.12 (-)	16.33 (-)

* Standard Deviation

** Threshold ChE activity below which samples are considered to have been exposed to a ChE inhibiting agent (Zinkl et al. 1980).

1 All data except Black and White and Wilson's warbler was tested for normal distribution using the Kolmogorov-Smirnov Goodness of Fit Test (α=.01 level of significance) and proved to be normally distributed. Black and white and Wilson's warbler provided too few samples to test.

2 There was no significant difference (ANOVA, α-.01) between the sample means of the white-throated sparrow, yellow-rumped warbler, blackpoll warbler and magnolia warbler. Black and white and Wilson's warbler provided too few samples to test.

TABLE 2.2: Brain activities (μ moles/min/g) of songbirds collected from forests sprayed with 210 g AI/ha fenitrothion

Species	Sample Size (n)			Mean, $\bar{X}$ (SD), [1,3]		
	Spray 1	Spray 2	Total Collection	Spray 1	Spray 2	Total Collection[2]
White-throated Sparrow	19	21	40	16.60 (7.62)*	18.52 (5.87)*	17.64 (6.74)*
Yellow-rumped Warbler	7	4	11	16.67 (7.94)*	22.99 (4.79)	19.03 (7.38)*
Magnolia Warbler	3	1	4	14.17 (5.82)*	25.76 (-)**	17.07 (7.49)*

1 All data was tested for normal distribution using the Kolmogorov-Smirnov Goodness of Fit Test and proved to be normally distributed (α=.01).

2 There was no significant differences (ANOVA, α=.01) between the total sample means of sprayed birds.

3 Magnolia warbler collected after Spray 2 could not be tested for normality.

* Sample mean significantly different (ANOVA, α=.01) than control sample mean of same species.

** Not tested for significant differences since 1 sample only.

whether or not they had received shot damage. Brain ChE activity was tested for differences based on undamaged and damaged brains within a species group (i.e. WTS unsprayed damage 0 vs WTS unsprayed damage 1 + 2 combined). Damaged brains had higher mean ChE activities than undamaged brains but of the 4 groups tested (WTS unsprayed, WTS spray 1, YRW unsprayed, YRW spray 1) only the YRW spray 1 group had a statistically significant higher ChE level (ANOVA, α=.01) when compared to YRW spray 1 undamaged brains.

2.2 Biological Significance

Whereas the tests for statistical significance among the data focused on grouping the ChE values and looking for differences, testing for biological significance focused on the ChE activity of individual birds.

Biological significance of ChE inhibition is shown in Table 2.3 by the number and percent of individual exposed birds exhibiting brain ChE activity 20%, 2 SD (standard deviations), and 50% lower than the mean ($\bar{X}$) ChE value for unsprayed birds of the same species. The threshold brain ChE activity below which samples are considered to have been exposed to a ChE inhibiting agent is the lower of the two values, unsprayed sample

TABLE 2.3: Number and percent of songbirds collected from forests sprayed with 210 g AI/ha fenitrothion that had ChE activity below the unsprayed sample mean ($\bar{X}$) - 2 SD, $\bar{X}$ - 0.2$\bar{X}$ and $\bar{X}$ - 0.5$\bar{X}$ of the same species.

	Sprayed Samples <Control $\bar{X}$ - 2SD			Sprayed Samples <Control $\bar{X}$ - 0.2 $\bar{X}$			Sprayed Samples <Control $\bar{X}$ - 0.5 $\bar{X}$		
Species	Spray 1	Spray 2	Total Collection	Spray 1	Spray 2	Total Collection	Spray 1	Spray 2	Total Collection
WTS	14(73.7%)	17(80.9%)	31(77.5%)	15(78.9%)	17(80.9%)	32(80.0%)	11(57.9%)	5(23.8%)	16(40.0%)
YRW	4(57.1%)	1(25.0%)	5(45.5%)	4(57.1%)	2(50.0%)	6(54.5%)	3(42.9%)	0(0.0%)	3(27.3%)
MAG	3(100.0%)	0(0.0%)	3(75.0%)	3(100.0%)	0(0.0%)	3(75.0%)	2(66.7%)	0(0.0%)	2(50.0%)
Total	21(72.4%)	18(72.0%)	39(70.9%)	22(75.9%)	19(76.0%)	41(74.5%)	16(55.2%)	5(20.0%)	21(38.1%)

WTS - White-throated Sparrow
YRW - Yellow-rumped Warbler
MAG - Magnolia Warbler

$\bar{X}$ - 0.2 $\bar{X}$ and $\bar{X}$ - 2 SD. These are standard test statistics used in brain ChE research to assess the biological impact of ChE inhibiting agents on individual birds.

The percent change in ChE activity in species groups exposed to fenitrothion spray relative to unsprayed species groups is shown in Table 2.4. Table 2.4 shows changes in mean group ChE values for birds of the same species in unsprayed and sprayed areas.

3.0 DISCUSSION

Statistically significant reductions in mean ChE activity in species of songbirds sprayed with 210 g AI/ha fenitrothion compared to mean ChE activity in unexposed groups of the same species indicate the spray application had an adverse effect on forest songbird populations in this study.

The tolerance of birds to and the biological significance of ChE depression is not fully understood (Busby et al. 1983). However, Ludke, Hill and Dieter (1975) suggested that 20% ChE inhibition of a sample when compared to the controls of the same species is enough of a depression to assume the bird was exposed a ChE inhibiting pesticide and that 50% inhibition could cause death. Zinkl et al. (1980) modified

TABLE 2.4: Percent change in brain ChE activity of species groups collected from forests sprayed with 210 g AI/ha fenitrothion relative to unsprayed species groups.

Species	% Change		
	Spray 1	Spray 2	Total
White-throated Sparrow	- 43.5	- 37.0	- 40.0
Yellow-rumped Warbler	- 41.3	- 19.5	- 33.4
Magnolia Warbler	- 54.6	- 17.4	- 45.3

this standard and considered whichever value was lower, the mean ChE activity of the unsprayed sample minus 20% or the mean of the unsprayed sample minus 2 SD, would indicate ChE inhibition.

In this study, 39 (70.9%) of the songbirds collected after the fenitrothion applications had ChE activities indicative of being exposed to a ChE inhibiting agent according to Zinkl et al. (1980). Twenty-one (38.1%) of the songbirds collected after spraying had a potentially lethal ChE inhibition according to the criterion of Zinkl et al. (1980). In comparison, the ChE activity of all samples collected in unsprayed areas showed only 1 sample (1.8%) below the level indicative of showing exposure to a ChE inhibiting agent according to Zinkl et al. (1980). This comparison again shows the considerable difference unsprayed sample ChE values and sprayed sample ChE values.

Busby (pers. comm.) suggested that brain sample mass has some effect on ChE activity measured. Data were analyzed from our study to investigate any possible relationship between brain ChE activity and brain mass. The analysis is not rigid and the mass of each brain collected was not recorded. However, the study team coded the apparent shot damage received with most of the brain samples collected. For each species group, undamaged (i.e. whole) brains were compared with brains that had received shot damage (i.e. not

whole). Results indicated that one sample group (damaged) showed a statistically significant increase in ChE activity over the undamaged samples of the same species group. All groups tested showed a higher ChE activity in damaged specimens over undamaged specimens. This relationship between brain mass and ChE activity is not understood (Busby pers. comm.) and future studies should consider this possible relationship. The use of damaged or partial brains could underestimate ChE inhibition effects when grouping data for statistical analysis.

An often cited disadvantage of the ChE technique used to assess exposure to insecticide is that collection of birds by shooting is biased toward those that are actively singing or more conspicuous and presumably less affected by the insecticide. The measured impact therefore would be considered a minimum one. However, in this study this bias was minimized because most singing had stopped late July since adult birds were feeding young. Some species of warblers were already starting to migrate during the latter part (August) of the field study.

Since some bird species had already initiated fall migrations and adverse affects of the spray operation may be underestimated due to birds moving through the area, sampling birds in a relatively small spray block (10 swaths) would also tend to underestimate the adverse effects since

affected birds could leave the area or birds outside the spray area could move into the study area after spraying operations are completed (D. Busby pers. comm.).

4.0 CONCLUSIONS

Based on the results of this study there is reason to be concerned about the effects of fenitrothion spraying operations at 210 g AI/ha on forest songbirds in Newfoundland. Evidence from this study suggests that potentially life threatening exposure to fenitrothion of some songbirds sampled (38.1%) had occurred according to the criterion of Zinkl et al. (1980).

Although this is a monitoring study the results from fenitrothion spraying events are largely descriptive of the individual spray event and evidence suggests that caution should be taken before making broad predictions of all fenitrothion spraying events from one monitored event. Fenitrothion spraying events are marginally safe for forest songbirds if the spray operations proceeds ideally (D. Busby, pers. comm.). Variable weather conditions can prevent a spray operation from being a near perfect event. The 1985 and 1986 songbird monitoring studies conducted by DFRL are a case in point. Both studies monitored the effects on songbird brain ChE after an operational spray

application of 210 g AI/ha fenitrothion. Strong and Wells (1985) determined that 7% of songbirds sampled were exposed to a potentially lethal dose of a cholinesterase inhibiting agent while in this study 38.1% of the songbirds collected were exposed to a potentially lethal dose of ChE inhibitor. The results from the Codroy Pond overspray incident (Addendum) show approximately 42.8% of the songbirds collected were exposed to a potentially lethal dose of a ChE inhibitor. Although the results of the Codroy overspray incident are quite similar to the results observed in the 1986 study, fenitrothion was accidentally applied at a rate of 350 AI g/ha (over 1.6 x the normal application rate).

These results reveal that it may not be possible to use the results of a single spray event monitoring study to reliably predict the impact of other spray events on forest songbirds. Songbird monitoring studies of fenitrothion spray events are useful for describing the adverse effects of the specific spray event.

The adverse effects of fenitrothion on forest songbirds are well documented (see Section 1.0) and forest managers should continue to monitor the potential adverse impact of fenitrothion on forest songbirds as part of their forest management programs.

5.0 RECOMMENDATIONS

In view of the measured adverse effect of fenitrothion applied at 210 g AI/ha on forest songbird brain ChE activity in this study, the DFRL should continue their research and development of forest insect control spray formulation and application rate. It is recommended that future monitoring efforts be initiated as early in the spray program as possible. The contract for this monitoring study was assigned on 11 July 1986 with the first spray delivered 27 July 1986. Spraying operations had been underway since early July on the west coast of insular Newfoundland. By the end of July in central Newfoundland some species of forest songbirds (magnolia warbler in particular) had already started to migrate. The collection of birds during migration could result in underestimating the effect of ChE inhibitors. Efforts should be made to develop a standard monitoring program to provide annually consistent collection methods to enable researchers to make valid comparisons and compilations of data.

Future monitoring programs should be more extensive, i.e. monitor more spray blocks in any spray regime, than intensively monitoring one spray event such as in this study. Monitoring more spray blocks would enable forest managers to better assess the overall impact of fenitrothion on songbirds.

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7.0 PERSONAL COMMUNICATIONS

D. Busby and P. Pearce, Canadian Wildlife Service, Fredericton, N.B.

APPENDIX A1

Songbird Brain Cholinesterase
Chemical Analysis

Analysis Performed By:

Canadian Wildlife Service
Environment Canada
P.O. Box 400
Fredericton, N.B.
E3B 4Z9

SPRAY BLOCK #107

ChE activities expressed as micromoles hydrolyzed/minute/g brain, wet weight.

Control	Spray 1	Spray 2
White-throated Sparrows		
33.14	30.25	23.65
26.63	32.16	18.90
30.25	11.17	32.16
34.65	27.07	17.04
25.33	13.17	20.83
26.19	14.54	17.04
27.96	12.83	20.43
32.16	7.38	24.06
28.86	17.78	9.55
33.14	17.41	14.54
35.16	9.24	21.62
32.16	9.87	15.24
20.83	14.54	17.41
31.20	16.68	8.92
29.78	14.54	18.15
24.48	8.30	15.60
30.25	27.07	22.02
28.41	10.19	13.17
31.40	22.42	17.04
23.23		29.78
31.68		11.82
29.78		
29.32		

SPRAY BLOCK #107

ChE activities expressed as micromoles hydrolyzed/minute/g brain, wet weight.

Control	Spray 1	Spray 2
Yellow-rumped Warblers		
30.72	24.90	27.51
27.07	18.52	22.02
31.20	23.65	16.68
22.02	24.48	25.76
31.20	9.55	
19.28	7.38	
27.51	8.92	
28.41		
28.86		
31.20		
21.22		
38.89		
32.16		
29.32		
29.32		

SPRAY BLOCK #107

ChE activities expressed as micromoles hydrolyzed/minute/g brain, wet weight.

Control	Spray 1	Spray 2
Blackpoll Warbler		
36.73	No Samples	No Samples
27.07		
29.78		
25.76		
42.90		
32.16		
35.16		
27.07		
28.41		
36.73		
48.55		
Magnolia Warbler		
28.41	8.92	25.76
36.20	13.17	
28.41	20.43	
33.14		
29.78		

SPRAY BLOCK #107

ChE activities expressed as micromoles hydrolyzed/minute/g brain, wet weight.

Control	Spray 1	Spray 2
Black-throated Green Warbler		
No Samples	No Samples	11.17
Black and White Warbler		
24.90 27.07	No Samples	No Samples
Wilson's Warbler		
32.65	No Samples	No Samples
Northern Waterthrush		
No Samples	25.76	No Samples

ADDENDUM

CODROY POND OVERSPRAY

Summary

During the 1986 hemlock looper control program one flight line in spray block #101 near Codroy Pond was sprayed with a fenitrothion mixture with more active ingredient than scheduled.

On 18 July, fenitrothion was applied at a rate of approximately 350 g AI/ha instead of the scheduled 210 g AI/ha. On the following day, 6 white-throated sparrows (WTS) and 1 blackpoll warbler (BP) were collected, their brains removed and prepared for analysis of ChE activity. Brain ChE activity was compared to unsprayed samples of the same species collected from spray block #107. Three (50%) of the WTS had brain ChE activity indicating exposure to a ChE inhibiting agent and 2 (33%) WTS had brain ChE activity indicating exposure to potential lethal dose of a ChE inhibiting agent.

The BP had brain ChE activity indicating exposure to a potential lethal dose of a ChE inhibiting agent.

Background

The purpose of this addendum is to report a fenitrothion overspraying incident and the results of brain cholinesterase (ChE) analysis conducted on forest songbirds collected in the area of the overspray.

The overspray incident occurred on 18 July 1986 in the morning during the spray application of fenitrothion to operational spray block #101. One flight line was sprayed with too much spray. Instead of 210 g AI/ha this flight line was thought to have received approximately 350 g AI/ha. In amount of spray, this equates to 1136 L (210 g AI/ha) being delivered on the flight line instead of the 681 L (210 g AI/ha) required. The location of the spray block is shown in Figure A1 (following page).

Fudge, Lane and Associates Limited (FLA) was requested to collect forest songbirds in the area of the overspray on 19 July 1986 to assess the impact on forest songbirds.

Study Team

The study team consisted of Mr. John Wells, Mr. Bruce Mactavish and Mr. Pierre Ryan (for details see Fudge, Lane and Associates Limited, 1986).

Acknowledgements

Fudge, Lane and Associates Limited would like to acknowledge the assistance of Mr. Paul Anderson, Department of Forest Land and Resources, during collecting.

Methods

Collection methods are similar to those previously described for the forest songbird monitoring project (Fudge, Lane and Associates Limited, 1986). Brain samples were analysed by the Canadian Wildlife Service, Fredricton, New Brunswick.

Results

Six white-throated sparrows (WTS) (Zonotricha albicollis) and one blackpoll warbler (BP) (Dendroica striata) were collected in the area of the oversprayed flight line.

The mean ($\bar{X}$) brain ChE value for white-throated sparrows was 19.23 μ moles/min/g with a standard deviation (SD) of 7.03.

The brain ChE value for the blackpoll warbler was 11.82 μ moles/min/g.

The results are shown in Table A.1 (following page).

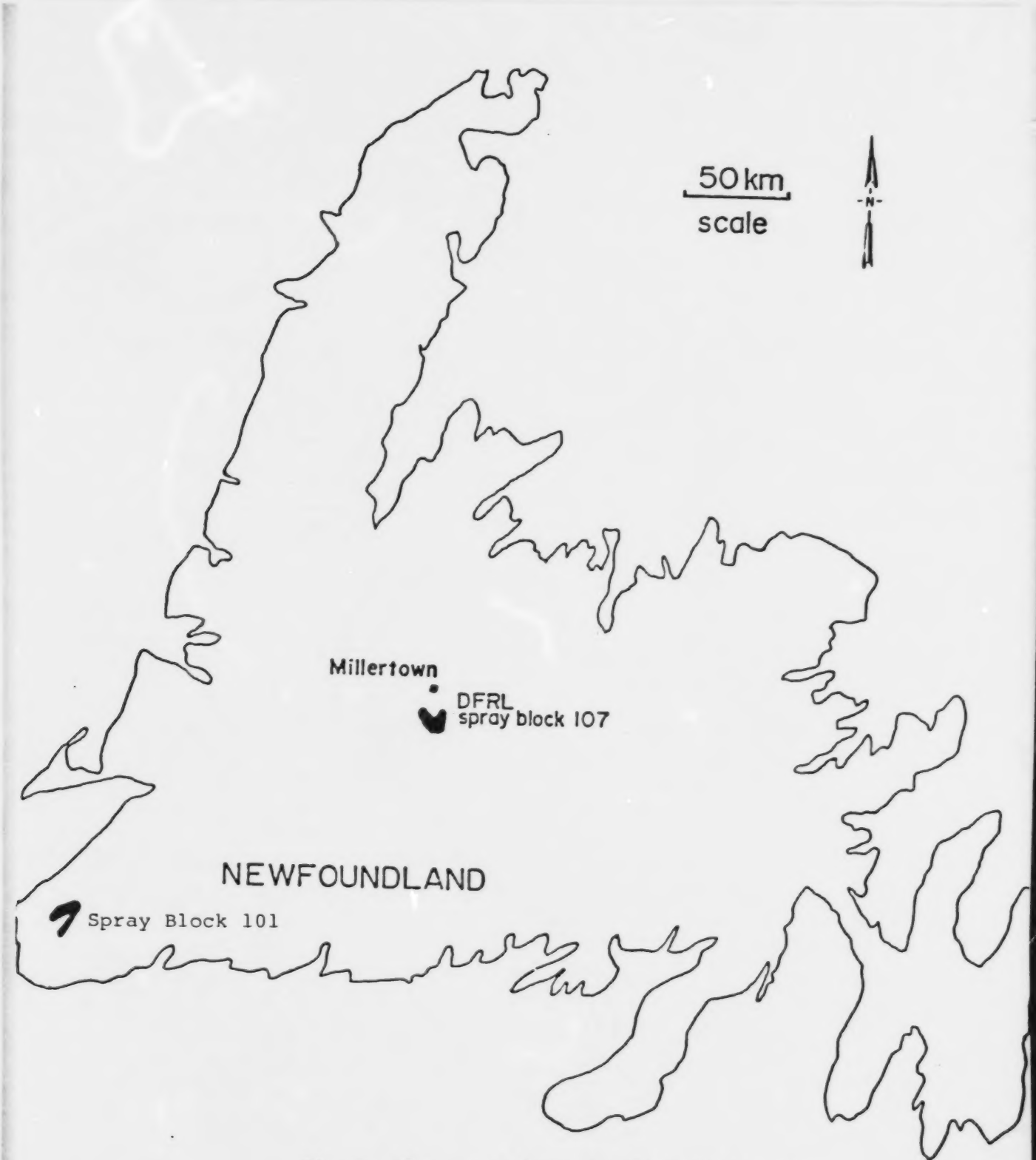

Figure A1 - Location of Spray Block 101

TABLE A.1: Brain ChE analysis* of samples collected in spray block #101 compared to unsprayed sample means ($\bar{X}$) and standard deviation (S.D.) of the same species collected from spray block #107

Species	Sample Size Block #101	Mean (SD) ChE Activity (μ mole/ min/g) Block #101	Sample Size Unsprayed Block #107	Mean (SD) ChE Activity (μ mole/ min/g) Block #107	# of Block #101 Samples Below Unsprayed Block #107 $\bar{X}$ - 2 S.D.** (% of Sample)	# of Block #101 Samples Below Unsprayed Block #107 $\bar{X}$ - .2 $\bar{X}$ (% of Sample)	# of Block #101 Samples Below Unsprayed Block #107 $\bar{X}$ - .5 $\bar{X}$ (% of Samples)
WTS	6	19.23 (7.03)	23	29.39 (3.64)	3 (50%)	4 (67%)	2 (33%)
BP	1	11.82 (-)	11	33.67 (7.23)	1 (100%)	1 (100%)	1 (100%)

* sample sizes from block #101 were not analysed from statistical significance but were analysed from biological significance according to the criterion of Zinkl et al. (1980).

** threshold ChE activity below which samples are considered to have been exposed to a ChE inhibiting agent (Zinkl et al. 1980).

Discussion and Conclusions

There wasn't opportunity to collect unsprayed birds in the Codroy Pond (#101) overspray area due to timing constraints. Brain ChE activities from samples collected at block #101 are compared to unsprayed samples of the same species collected from block #107 where the songbird monitoring study occurred. Statistical analysis of the Codroy Pond samples was not performed due to small sample size. However, comparisons based on the biological significance (see Fudge, Lane and Associates Limited, 1986, Zinkl et al. 1980) of the ChE activity values between samples from block #101 and #107 were made.

Three (50%) WTS collectd in block #101 had brain ChE activity below $\bar{X}$ - 2 SD of the unsprayed WTS sample collected at block #107. Four (67%) WTS collected at block #101 had brain ChE activity below $\bar{X}$ - .2 $\bar{X}$ of the unsprayed WTS sample collected at block #107. Two (33%) WTS collected at block #101 had brain ChE activity below $\bar{X}$ - .5 $\bar{X}$ of the unsprayed WTS sample collected at block #107.

Based on these comparisons, indication of exposure of a brain ChE inhibiting agent had occurred in at least 50% of the WTS collected in block #101 when compared to mean brain ChE activity of unsprayed WTS in block #107.

Thirty-three percent of the WTS collected in block #101 compared to unsprayed WTS samples from block #107, indicated exposure to a potential lethal dose of a brain ChE inhibiting agent (criterion of Zinkl et. al. 1980).

The BP sample collected in block #101 when compared to unsprayed BP samples collected from block #107, indicated exposure to a potential lethal dose of a brain ChE inhibiting agent (criterion of Zinkl et al. 1980).

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STUDY TEAM

The study team consisted of the following personnel:

Stephen M. Fudge (M.Sc.), Biologist - Project Manager

John Wells (B.Sc.), Biologist - Field Supervisor

Mr. Wells has over 18 years experience as a bird watcher and is able to identify all birds in Newfoundland by sight and song. He was field supervisor for the 1985 songbird monitoring study. His extensive field experience and knowledge of avian biology as well as his supervisory experience ensured quality data collection in the field. Mr. Wells assisted in the report preparation.

Bruce Mactavish, Wildlife Technician

Mr. Mactavish assisted Mr. Wells in the collection of field data. Mr. Mactavish is an internationally recognized expert on Newfoundland avifauna. He is a competent woodsman and has superior field talents in bird finding and identification.

Pierre Ryan (B.Sc.), Wildlife Biologist

Mr. Ryan assisted Mr. Wells in the collection of field data. Mr. Ryan has an extensive background in avifaunal studies. He has been involved in bird banding and survey projects for the

Canadian Wildlife Service in Newfoundland and Labrador for the past 6 years.

David Robbins (B.Sc. Pending), Biologist

Mr. Robbins assisted Mr. Wells in gathering field data. Mr. Robbins has field experience in several fields of biology and is also a competent shooter and woodsman.

David Lemon (B.Sc., B.Ed.), Biologist

Mr. Lemon coordinated the field aspect of this project, assisted in data interpretation and was responsible for the report preparation.

A STUDY TO MONITOR THE EFFECTS OF AERIALLY APPLIED FENITROTHION ON THE ACTIVITY OF NEWFOUNDLAND POLLINATING INSECTS

Prepared by

Fudge, Lane and Associates Limited

under contract to

the Minister of Forest Resources and Lands

for

the Minister of Environment

1987

The attached study was submitted to the Minister of Environment by the Minister of Forest Resources and Lands as required under the Pesticides Operator License issued by the Minister of Environment for the 1986 Hemlock Looper Forest Protection Program.

The Minister of Environment brought together an expert committee to review the technical merit of this study and found it to be inconclusive.

A STUDY TO MONITOR THE EFFECTS OF AERIALLY APPLIED FENITROTHION ON THE ACTIVITY OF NEWFOUNDLAND POLLINATING INSECTS

PREPARED FOR:

NEWFOUNDLAND AND LABRADOR DEPARTMENT
OF FOREST RESOURCES AND LANDS
CORNER BROOK, NEWFOUNDLAND

PREPARED BY:

FUDGE, LANE & ASSOCIATES LIMITED
ST. JOHN'S, NEWFOUNDLAND

DECEMBER 1986
F-109

EXECUTIVE SUMMARY

The Newfoundland and Labrador Department of Forest Resources and Lands (DFRL) conducted an aerial spray program to control an outbreak of the eastern hemlock looper (Lambdina fiscellaria fiscellaria) in insular Newfoundland during the summer of 1986. The insecticide used in the spray application was fenitrothion.

The DFRL was directed by the Newfoundland Department of Environment (NODE) to undertake a monitoring study to assess the impact of fenitrothion on insect pollinator populations.

The fenitrothion monitoring study area was located in, and adjacent to the DFRL spray block #107 near Harpoon Brook, approximately 20 km south of Millertown, Newfoundland. The study area is within the Central Newfoundland Ecoregion (Damman 1983). Much of the study area was cut over in the mid to late 1960's. Now the area is primarily composed of immature black spruce (Picea mariana) and balsam fir (Abies balsamea) stands. There are some remaining areas of mature black spruce. Some areas were not sufficiently restocked with trees after cutting operations were completed, according to Forest Inventory Maps.

The methodology chosen for the monitoring study was as follows:

Native Pollinator Activity

- activity of insect pollinators was assessed along a series of 100 m long transects in both spray and control areas. This was done through the comparison of standardized abundances. Observations were made daily over the period July 17 to August 5, 1986.

- sweep net insect collections were made to obtain reference collections and to collect weekly information on total bee and non-bee numbers.

Fruit Set Study

- buttercups, Ranunculus spp., were selected to compare fruiting success between control and treatment blocks. Twenty-five plants per transect were tagged and monitored prior to and subsequent to spraying.

Results were analyzed and compared with the literature, particularly the 1985 Hemlock Looper Spray program study.

Results

The study team was in the field from July 17 to August 5, 1986.

Specimens of non-bee pollinators representing 28 families and a single family of bees (Apidae) were collected in this study. A total of 14 taxa of plants were recorded and used in the calculation of bloom abundance and bloom index (B.I.).

Mean transect B.I. values ranged between 3.0 and 3.8. Standardized abundances of bees and non-bee pollinators were not significantly correlated with B.I.

Abundances of pollinating insects varied greatly along the study transects over the course of the study. Coefficients of variation ranged from 3 to 173%.

Comparison of bee abundance data before and after spraying produced inconclusive results. However, there were definite trends towards a reduction in total insect numbers (primarily non-bee pollinators) subsequent to both spray applications in the treatment transects. Unfortunately, decreased abundances after first spray application were masked by a corresponding significant drop in air temperature.

Results of weekly net sweep trials revealed no significant differences in mean standardized bee and non-bee pollinator counts between control and treatment areas either before or after spray application.

Results of the fruit set study revealed no significant differences in mean abundances of buds, flowers or fruits between spray and control areas either before or after spraying.

Conclusions

Results of the present study revealed trends towards a reduction in total insect numbers subsequent to aerial spraying of fenitrothion. Results of bee abundance data prior to and subsequent to spraying were inconclusive.

Recommendations

Results of this monitoring study and previous studies have revealed that monitoring methodologies currently employed are unsatisfactory in terms of producing standardized results. In addition, the data produced in both the 1985 & 1986 studies were highly variable, possibly indicating problems with the methodology.

Major problems in the field include lack of control over actively moving pollinators such as bees, lack of knowledge on fruiting periods of many Newfoundland plants and the interaction between pollinating insects and the various flowering plant species.

It is recommended that current monitoring methodology for assessing pollinator activity be re-evaluated for improvement or total revision. This would allow standardized methodology to be incorporated into the terms of reference.

Additionally, it is recommended that the usefullness of the fruiting success component study be re-evaluated. In this years study, it was apparent that some plants had already fruited prior to spray application.

Study Team

Mr. Stephen M. Fudge, M.Sc.

Mr. Fudge was project manager and co-ordinator for this project. Mr. Fudge has had exposure to a wide range of resource inventory and analysis projects including forest insect control programs in New Brunswick, Maine and Newfoundland.

Mr. John Pratt, B.Sc.

Mr. Pratt was the field co-ordinator for the project. He is a terrestrial biologist with an extensive knowledge of native flora. Mr. Pratt verified identification of plant specimens.

Valerie Anstey, B.Sc., Sandra Meron, B.Sc., Barry Hicks, B.Sc. (pending)

These biologists conducted the fieldwork for this project. All three have academic backgrounds in entomology and botany.

Mr. Kent Gilkinson, M.Sc.

Mr. Gilkinson prepared this report.

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1.0 INTRODUCTION

The Department of Forest Resources and Lands (DFRL) was directed to undertake a monitoring study of the effects of the 1986 hemlock looper spray (fenitrothion) program on insect pollinators in Newfoundland. Presently, the role of insect pollinators in Newfoundland ecosystems is not well known (Larson et al. 1983). However, it is known that many plants require insect pollinators for successful seed and fruit formation. Seed and fruit can be seriously reduced if pollinator populations, particularly bees, are low (Larson et al. 1983). Lomond and Larson (1983) discovered that by augmenting the native bee population in Newfoundland coastal barrens, a significant increase (25%) in blueberry (Vaccinium angustifolium) production could be realized.

In 1970 several New Brunswick blueberry growers, whose fields were located near fenitrothion sprayed forests, reported unusually low pollinator activity and attributed their crop losses to the spray operation (Wood, 1982). Subsequent studies confirmed that there is significant mortality of native bees in forests sprayed with fenitrothion (Plowright and Rodd, 1980; Plowright et al. 1978; Thorpe, 1979; Kevan, 1975; Wood, 1977). Both domestic and native bees are very susceptible to many chemical insecticides and wild bee populations have been seriously depleted in many areas where agricultural chemicals are used extensively (Plowright and Rodd, 1980).

In order to monitor the effects of fenitrothion on insect pollinators and to analyze pollinator success in 1986, one control area (unsprayed) and one spray block in central Newfoundland were monitored. The study attempted to identify and describe any effects of aerially applied fenitrothion (Table 1.1) on pollinators and pollinator success by using bloom abundance to calculate a bloom availability index (B.I.) and by counting the number and type of insect pollinators on blooms and subsequent pollinator success through fruit set.

2.0 STUDY AREA

The study area was located about 20 km south of Millertown, Newfoundland (Figure 2.1), within the central Newfoundland Ecoregion (Damman, 1983). The area is characterized by balsam fir (Abies balsamea) and black spruce (Picea mariana) mature forests. Stands of trembling aspen (Populus tremuloides) and white birch (Betula papyrifera) were also found in the study area. Scrub areas of aspen and alder (Alnus rugosa) and regenerated cut-over areas occur in the study area. Dwarf shrub heath dominated by Kalmia angustifolia and small bog areas consisting of Sphagnum spp. occur locally.

Much of the study area was cut-over in the mid-to late 1960's. The regenerated areas are composed of immature (0-20 yrs.) black spruce and balsam fir under 3-5 m in height and some mature black spruce (41-60 and 61-80 yrs.) 9-12 m high. Some areas were not sufficiently restocked (DFRL Forest Inventory Maps).

3.0 MATERIALS AND METHODS

3.1 Experimental

Spray block 107 was located east of Harpoon Brook. Spraying was conducted along 10 N-S lines; spray paths are illustrated in Figure 3.1. Four 100 m treatment transects (A-D) were placed back to back running N-S along the roadside (photos 1-4). These transects were to the left of spray line #2.

3.2 Control

The control study area was located outside of spray block 107. Three 100 m transects (F-H) were positioned along the Harpoon Camp road north of center block 107 (photos 5-8). The fourth transect (E) was located west of Harpoon Brook dam, adjacent to the road.

TABLE 1.1 Technical details of spray application for pollinating insect monitoring study (1986).

Spray Application

Aircraft:	DC-4
Aircraft Height:	61 m
Aircraft Speed:	305 km/hr.
Aircraft Apparatus:	Boom and nozzle with #8010 spray tips

Spray Mixture

Insecticide:	Fenitrothion (Folithion^{R})
Mixture (by volume):	11% fenitrothion
	40% cyclosol 63
	49% ID 585 (insecticide diluent)

Application Rate: 210 g active ingredient/1.5 l/ha (two applications)

Application Dates: 1st. spray - 27 July (evening)
2nd. spray - 2 August: partial (evening)
3 August: completed (morning)

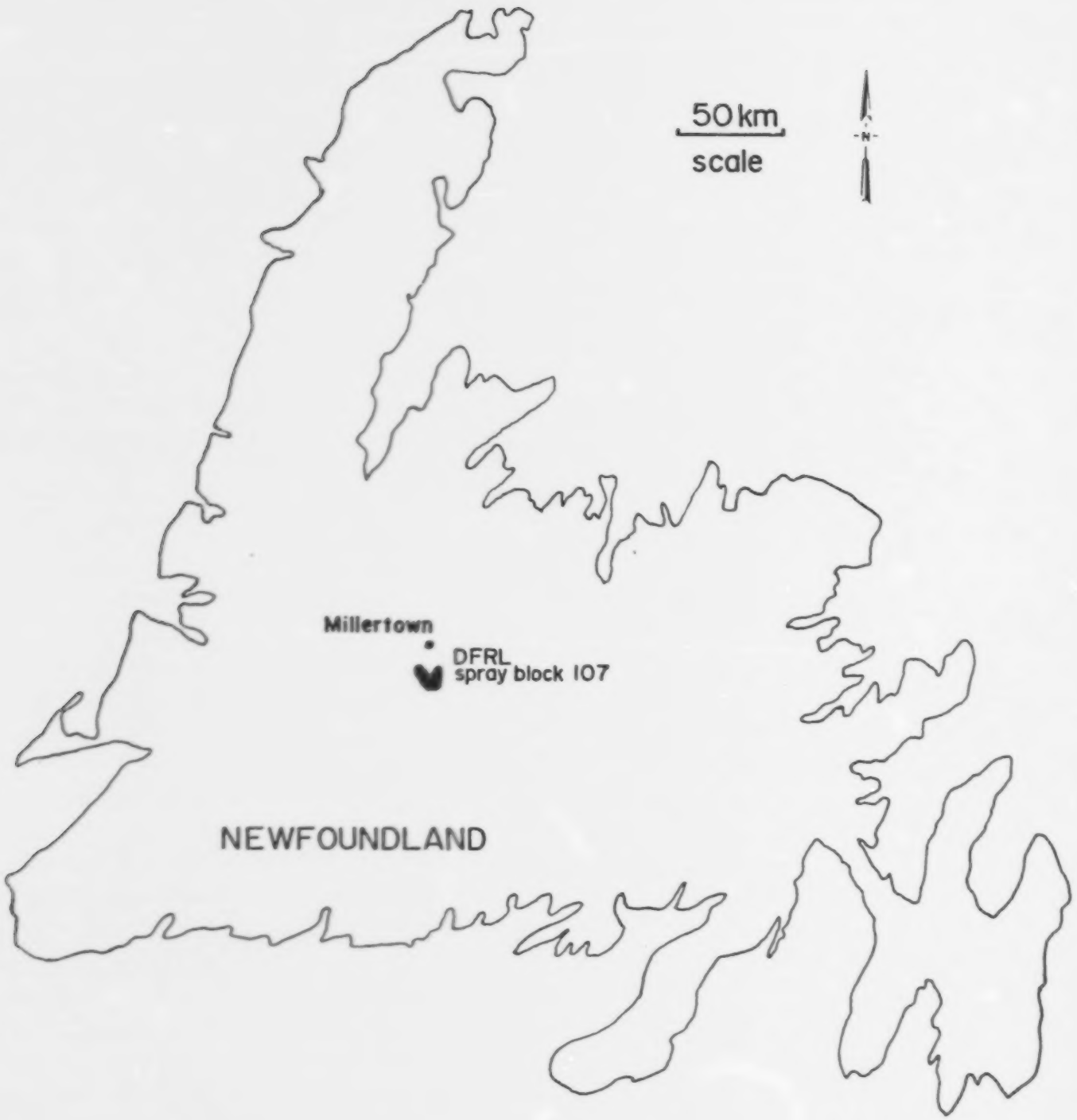
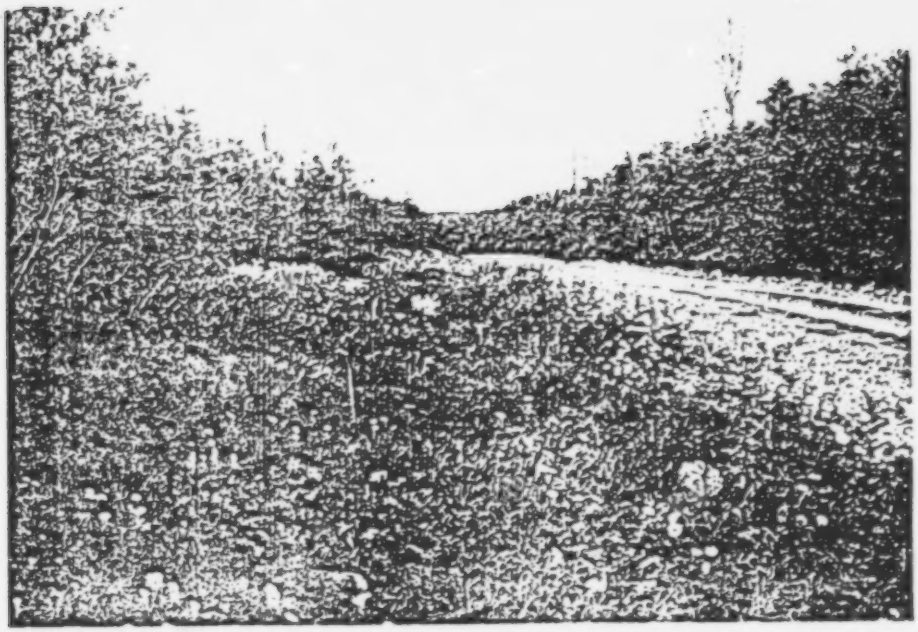


Figure 2.1. Location of study area.

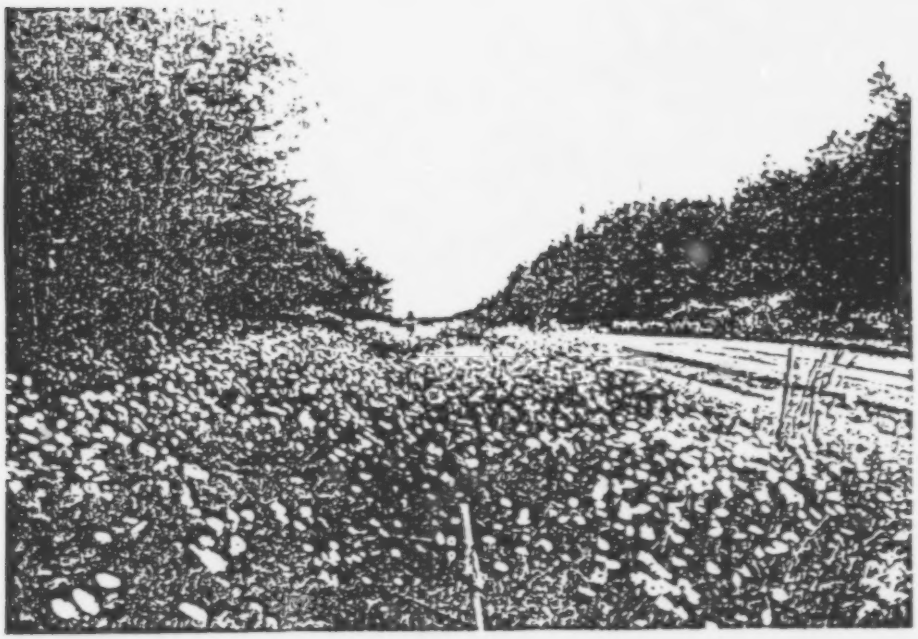
Figure 3.1. Location of study area. Spray lines are indicated by stipled lines. Discontinued spraying is indicated by jagged lines.

△ - control transects E-H

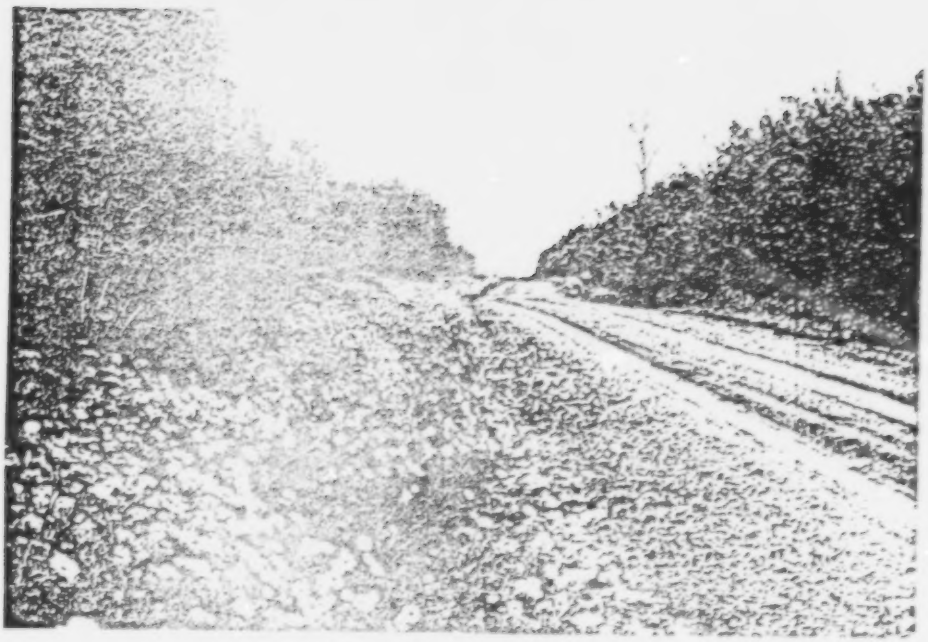
▲ - treatment transects A-D



Photograph 1. Location of treatment transect "A".

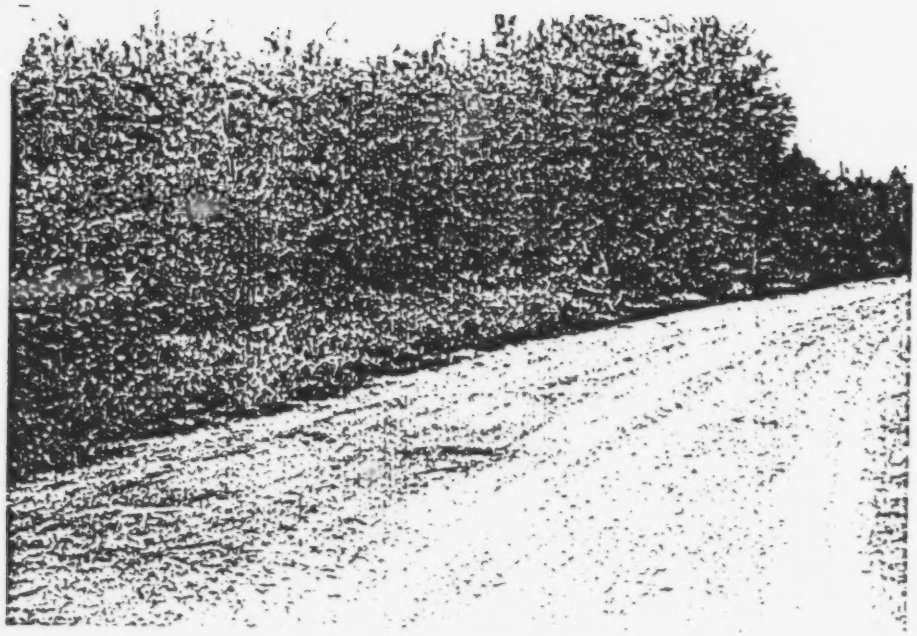


Photograph 2. Location of treatment transect "B".



Photograph 3. Location of treatment transect "C".

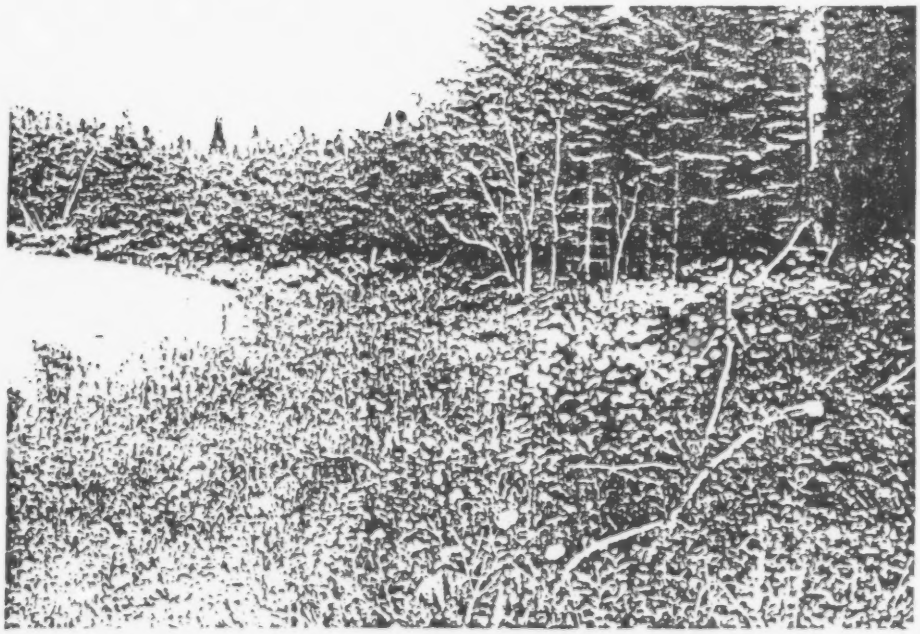
Photograph 4. Location of treatment transect "D".



Photograph 5. Location of control transect "E".

Photograph 6. Location of control transect "F".

Photograph 7. Location of control transect "G".



Photograph 8. Location of control transect "H".

3.3 Native Pollinator Monitoring

Insect pollinators were, in most cases, identified to the level of family and categorized as either bee (family Apidae) or non-bee pollinators. In order to assess native pollinator activity, each 100 m transect in both spray and control areas was divided into 10 m segments. An observer walked a transect spending exactly 5 minutes in each section recording total number and type of pollinator (bee vs. non-bee).

Observations were made daily (with a few exceptions) over the period July 17 to August 5, 1986. Each 10 m segment of tansect was assessed a bloom abundance value based upon a ranking scheme (Table 3.1).

Bloom abundance data were then used to calculate bloom index (B.I.) values. The B.I. value for a transect on a given day is the sum of the bloom abundance values (0 to 5) for all 10 m segments of the transect, divided by 10 (the total number of segments per tansect). In order to make comparisons of pollinator populations, counts were standardized by dividing the number of pollinating insects observed, by the transect B.I. value (Larson et al. 1986). This produced a measure of the abundance of insect pollinators per uniform bloom unit. These data provided the basis for comparison of insect pollinator populations from areas subjected to aerial spraying and those non-sprayed areas used as controls. Other transect data collected included:

- time of commencement and termination of observations;
- weather: temperature and wind strength (Beaufort Scale);
- exposure to the sun (full exposure, partly shaded, completely shaded).

TABLE 3.1 Bloom abundance scale used to assess pollinator activity.

Value Assigned	# Plants Blooming per 10 m Transect Section
0	0
1	1
2	2 - 5
3	6 - 15
4	16 - 25
5	>25

Transects were "walked" daily for 7 days (July 17 to July 27) prior to first spray application. The first spray application was made on July 27. Due to the collection of residue samples monitoring commenced again on July 29 to August 2. The second spray application was started on the evening of August 2 and was completed on the morning of August 3. Monitoring was then conducted over the next three consecutive days.

3.4 Sweep Net Insect Collections

Timed sweep-net collections of insects from flowers were made weekly during the periods before spray, subsequent to first spray and subsequent to second spray applications. Net sweep data were collected to obtain insect pollinator reference collections and to collect weekly information on total bee and non-bee numbers. Collections were made with sweeps lasting 2-3 minutes per 10 m segment of transect with the entire 100 m of each transect being swept. The sweeps were undertaken in such a manner as to leave the plants intact. The nets were gently swept over blooms collecting insects on plants and in the air immediately over them. The insects were then placed in killing jars containing ethyl acetate. The collections were later sorted, then emumerated. Representative specimens were then dry preserved, pinned and labelled.

3.5 Fruit Set Study

The fruit set study was conducted on a weekly basis. Data was collected prior to spraying, subsequent to first spray and subsequent to second spray application. Buttercup, Ranunculus spp., were selected for this aspect of the study. Buttercup was chosen because it was in flower whereas the majority of the blueberry (Vaccinium angustifolium) and raspberry (Rubus idaeus) flowers had already set fruit by the time the study commenced.

Twenty-five buttercups per transect were tagged for subsequent monitoring. Each plant was examined for the presence of bud, flower and/or fruit and total counts of each were made.

4.0 RESULTS

4.1 Pollinator Activity Monitoring

Plants used to estimate bloom abundances and to calculate B.I. values are shown in Table 4.1. Insect pollinators collected are shown, by major category, in Table 4.2. Tables 4.3 and 4.4 summarize data on bloom and pollinator abundances for each transect within treatment (spray) and control study areas over the course of the study.

Mean B.I. values ranged between 3.1 and 3.8 over the course of the study. The overall, mean B.I. was 3.46 ± 0.24 for treatment transects and 3.36 ± 0.12 for control transects. Mean B.I. values differed significantly between transects within the treatment block (ANOVA: $p<0.05$) while there was no significant difference between transects in the control area. Mean B.I. values did not differ significantly between control and treatment areas prior to or subsequent to spraying.

Standardized abundances of bees and non-bee pollinators were not significantly correlated with B.I. values ($r=0.2$ and 0.09 respectively) as determined with pre-spray data. Although there was an absence of correlation, abundances of pollinators were standardized to conform to previous studies. A common feature of the data throughout is the large variation in counts of pollinating insects (coefficients of variation ranged from 3 to 173%).

TABLE 4.1 Species of plants used to calculate bloom abundance and B.I. values along study transects.

Species	Common Name
Lamium album	White dead nettle
Trifolium repens	White clover
Chrysanthemum leucanthemum	Ox-eye daisy
Rumex acetosella	Common sorrel
Euphrasia americana	Eyebright
Vicia cracca	Cow's vetch
Ranunculus spp.	Buttercup
Hieracium aurantiacum	Orange hawkweed
H. pratense	Yellow hawkweed
Prunella vulgaris	Heel-all
Leontodon antumnalis	Autumn dandilion
Anaphalis margaritacea	Pearly everlasting
Achillea Millefolium	Yarrow
Epibolium angustifolium	Fireweed

TABLE 4.2 Major groups of pollinating insects identified in this study.

Bees

Apidae

Non-Bees

Acrididae
Apocrita
Cantharidae
Cerambycidae
Cercopidae
Chrysididae
Chrysopidae
Cicadellidae
Coccinellidae
Coenagrionidae
Colletidae
Corimelaenidae
Diptera
Formicidae
Halictidae
Hemiptera
Homoptera
Hymenoptera
Icheneumonidae
Lepidoptera
Miridae
Nymphalidae
Sphecidae
Stratiomyidae
Syrphidae
Tabanidae
Tipulidae
Vespidae

TABLE 4.3 Means of bloom indices and abundances of pollinating insects within each study transect prior to first spray application.

Transect		Observ.	$\bar{X}$ B.I.	$\bar{X}$ Non-Bees	$\bar{X}$ Bees	$\frac{\bar{X}\text{ Non-Bees}}{\bar{X}\text{ B.I}}$	$\frac{\bar{X}\text{ Bees}}{\bar{X}\text{ B.I.}}$
Treatment	A	7	3.8	95.71 ± 38.33	7.14 ± 9.56	25.18	1.88
	B	7	3.6	94.43 ± 34.43	10.14 ± 15.95	26.23	2.82
	C	7	3.5	112.00 ± 42.08	8.86 ± 9.75	32.00	2.53
	D	7	3.2	94.86 ± 33.77	10.00 ± 12.00	29.64	3.13
Control	E	7	3.3	60.57 ± 30.39	12.43 ± 13.28	18.35	3.77
	F	7	3.3	65.00 ± 22.72	4.00 ± 1.63	19.69	1.21
	G	7	3.4	84.86 ± 34.53	3.14 ± 3.13	24.96	0.92
	H	7	3.7	82.43 ± 31.00	6.86 ± 4.91	22.28	1.85

TABLE 4.4 Means of bloom indices and abundances of pollinating insects within each study transect subsequent to first spray application.

Transect		Observ.	$\bar{X}$ B.I.	$\bar{X}$ Non-Bees	$\bar{X}$ Bees	$\frac{\bar{X}\text{ Non-Bees}}{\bar{X}\text{ B.I}}$	$\frac{\bar{X}\text{ Bees}}{\bar{X}\text{ B.I.}}$
Treatment	A	3	3.7	28.67 ± 32.35	6.00 ± 10.39	7.75	1.62
	B	3	3.5	29.33 ± 27.79	6.67 ± 10.69	8.38	1.91
	C	3	3.5	35.00 ± 49.43	7.33 ± 11.85	10.00	2.10
	D	3	3.1	20.67 ± 28.01	3.67 ± 6.35	6.67	1.18
Control	E	3	3.3	22.00 ± 27.73	7.67 ± 8.96	8.40	2.32
	F	3	3.4	54.33 ± 19.55	2.00 ± 2.65	15.98	0.59
	G	3	3.4	48.67 ± 12.66	1.33 ± 2.31	14.31	0.39
	H	3	3.5	33.67 ± 16.80	2.67 ± 4.62	9.62	0.76

Prior to spraying there was no significant difference ($p>0.05$) in mean standardized number of bees between control (1.94) and treatment (2.59) areas (Table 4.3). However the mean standardized number of non-bee pollinators differed significantly ($p<0.05$) between control (21.32) and treatment areas (28.26) (Table 4.3). Subsequent to first spray application there was no significant difference ($p>0.05$) in mean standardized number of bees and non-bees between control and treatment areas (Table 4.4). However, subsequent to the second spray application both standardized number of bees and non-bees were significantly ($p<0.05$) lower in the treatment area vs. the control area (Table 4.5). This was reflected in the analysis of standardized abundances of total pollinators over the course of the study within each study transect. Results are presented graphically in Figures 4.1 and 4.2.

Prior to spraying, abundances did not differ significantly (ANOVA:$p>0.05$) within treatment and control areas (e.g. between transects). Subsequent to first spray application abundances dropped significantly in both treatment (Figure 4.1) and control (Figure 4.2) areas. However, this coincided with a significant drop in temperature (Figure 4.3). Subsequent to second spray application abundances dropped significantly in the treatment transects and increased significantly in the control area. Although there was a corresponding drop in temperature at this time also, it was not as marked as at the time of first spray application.

4.2 Net Sweep Data

Results of the weekly net sweep trials are summarized in Table 4.6). There were no significant differences ($p>0.05$) in mean standardized bee and non-bee pollinator counts between control and treatment areas either before or after spray application.

TABLE 4.5 Means of bloom indices and abundances of pollinating insects within each study transect subsequent to second spray application.

Transect		Observ.	$\bar{X}$ B.I.	$\bar{X}$ Non-Bees	$\bar{X}$ Bees	$\frac{\bar{X}\text{ Non-Bees}}{\bar{X}\text{ B.I}}$	$\frac{\bar{X}\text{ Bees}}{\bar{X}\text{ B.I.}}$
Treatment	A	3	3.6	37.67 ± 34.82	4.67 ± 6.43	10.46	1.30
	B	3	3.3	37.00 ± 34.12	3.00 ± 4.36	11.21	0.91
	C	3	3.4	19.33 ± 9.07	2.67 ± 2.89	5.68	0.79
	D	3	3.0	16.33 ± 11.55	1.67 ± 1.15	5.44	0.56
Control	E	3	3.3	62.33 ± 2.08	13.67 ± 11.59	18.88	4.14
	F	3	3.6	163.00 ± 83.36	14.67 ± 9.50	45.28	4.08
	G	3	3.3	139.33 ± 63.81	6.67 ± 3.79	42.22	0.20
	H	3	3.1	107.67 ± 55.64	7.67 ± 8.33	34.70	2.47

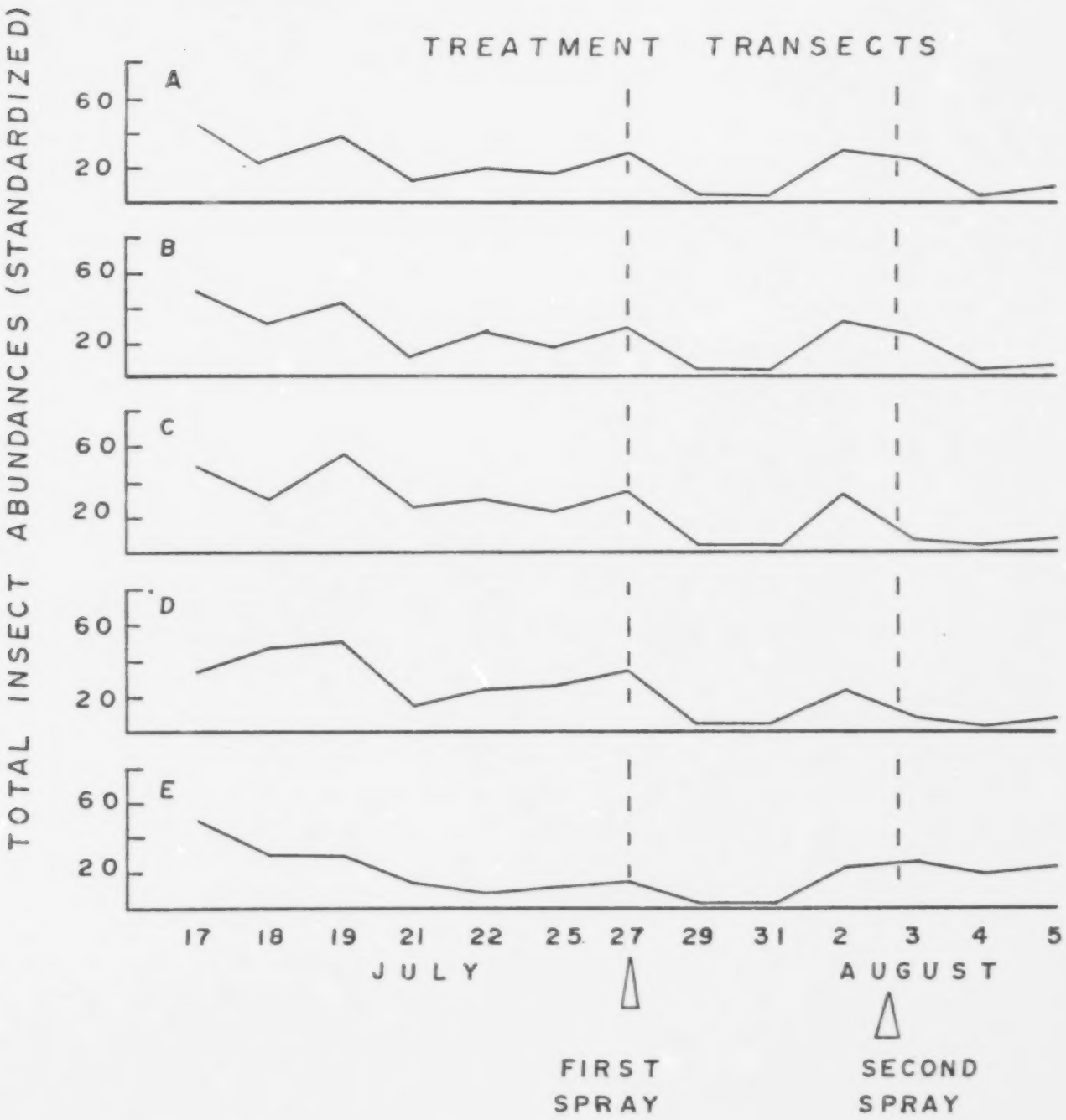


Figure 4.1. Total standardized insect abundances over treatment transects.

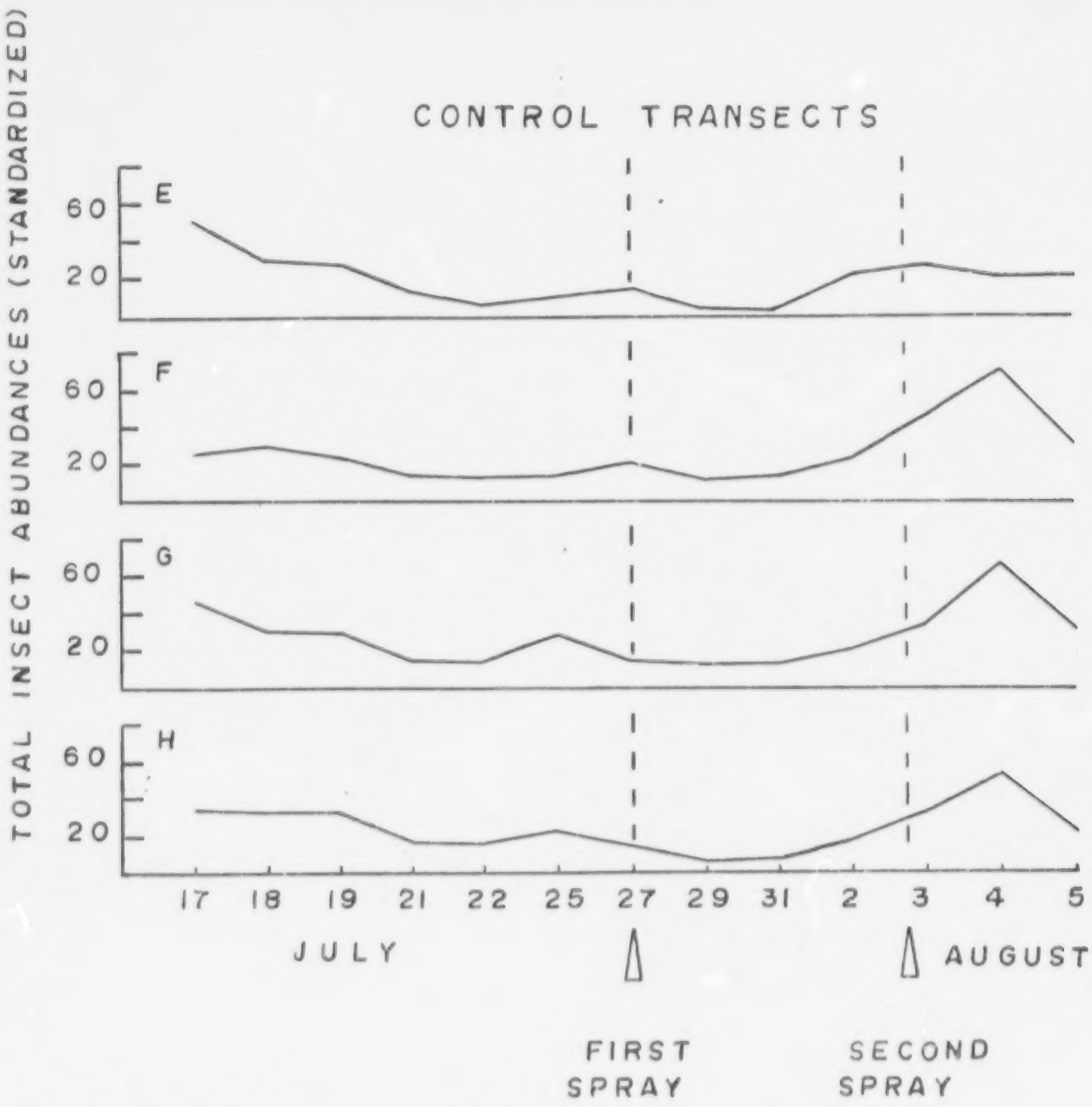


Figure 4.2. Total standardized insect abundances over control transects.

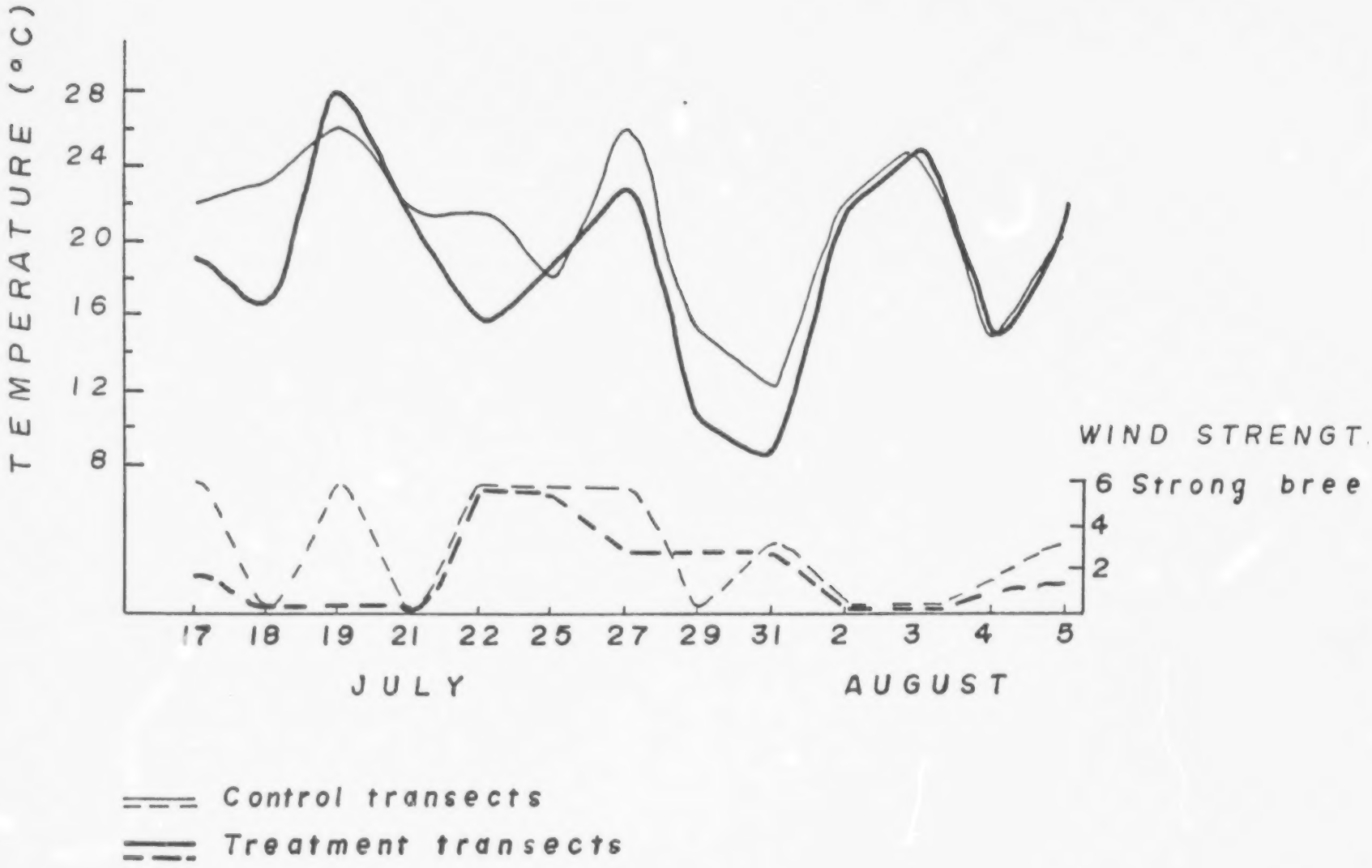


Figure 4.3. Temperature/wind profile within the study area.

TABLE 4.6 Standardized abundances of pollinating insects within study blocks from net sweeps.

	Total Pollinators			Bees			Non-Bees		
Study Block	Before #/B.I.	1st. Spray #/B.I.	2nd. Spray #/B.I.	Before #/B.I.	1st. Spray #/B.I.	2nd. Spray #/B.I.	Before #/B.I.	1st. Spray #/B.I.	2nd. Spray #/B.I.
Treatment (n=4)	80.02 ± 26.92	12.72 ± 7.07	27.19 ± 15.38	1.34 ± 1.95	0	1.20 ± 1.20	76.68 ± 26.62	12.72 ± 7.07	25.99 ± 15.25
Control (n=4)	46.67 ± 10.32	24.50 ± 8.81	38.18 ± 10.93	0.65 ± 0.26	0	2.50 ± 2.38	46.02 ± 10.31	24.50 ± 8.81	35.68 ± 9.11

Within the treatment area mean standardized total pollinator abundances dropped significantly after the first spray application. This was attributed to a significant decrease in mean abundance of non-bee pollinators.

In the control area, mean standardized total pollinator abundances were similar prior to spraying and after each spray period although mean standardized bee abundance dropped significantly after the first spray period.

It is not appropriate to compare pollinator abundances obtained from net sweeps vs. plant monitoring as the two methodologies differ.

4.3 Fruit Set

There were no significant differences in mean abundances of buds, flowers or fruits between spray and control areas either before or after spraying (Table 4.7).

5.0 DISCUSSION

5.1 Pollinator Activity Monitoring

A variety of factors could account for the absence of correlation between standardized abundances of bee and non-bee pollinating insects and bloom index. Both categories of insects are known to exhibit flower preferences (Larson *et al.* 1986). Potential variation in the quantity of specific bloom species within and between transects could thus be a contributing factor. Larson *et al.* (1986) found that while there was a correlation between standardized abundances of bees and bloom index, the distribution of non-bee insects (a very heterogenous group) varied independently of the quantity and perhaps, type,

TABLE 4.7 Mean bud (B), flower (Fl) and fruit (Fr) counts from 25 selected stems per transect in each study block.

		TREATMENT BLOCK								
		Before (July 16)			1st. Spray (July 31)			2nd. Spray (Aug. 4)		
Transect		B	Fl	Fr	B	Fl	Fr	B	Fl	Fr
Treatment	A	4.72 ± 2.37	3.32 ± 1.77	4.00 ± 2.66	1.55 ± 1.87	1.73 ± 1.49	9.52 ± 4.69	0.86 ± 1.17	1.09 ± 1.11	10.29 ± 4.31
	B	3.88 ± 2.21	3.38 ± 2.16	3.40 ± 2.82	1.07 ± 1.21	1.50 ± 1.61	7.87 ± 4.58	1.00 ± 1.24	0.44 ± 0.70	8.37 ± 4.39
	C	8.96 ± 5.58	3.68 ± 1.97	3.91 ± 2.71	3.59 ± 3.16	3.27 ± 3.10	10.73 ± 4.56	3.96 ± 4.56	1.83 ± 2.06	11.87 ± 7.50
	D	6.60 ± 6.71	2.88 ± 1.62	2.77 ± 2.41	4.05 ± 5.65	1.86 ± 2.17	7.09 ± 4.22	2.00 ± 3.31	1.29 ± 2.49	8.31 ± 5.79
		CONTROL BLOCK								
Control	E	4.76 ± 2.93	3.40 ± 1.58	3.38 ± 2.30	1.38 ± 0.97	1.29 ± 1.37	9.38 ± 3.87	1.17 ± 1.05	0.46 ± 0.66	9.52 ± 3.92
	F	5.60 ± 3.63	3.88 ± 2.55	1.72 ± 1.99	1.96 ± 2.13	0.96 ± 1.27	7.92 ± 4.66	0.92 ± 1.41	0.76 ± 1.09	8.12 ± 4.80
	G	3.52 ± 3.92	3.32 ± 1.49	2.54 ± 1.61	0.63 ± 0.97	1.08 ± 1.61	7.25 ± 2.71	0.43 ± 0.79	0.43 ± 0.90	7.57 ± 3.10
	H	5.60 ± 3.64	3.12 ± 1.81	2.18 ± 3.21	1.59 ± 1.82	2.00 ± 2.37	6.68 ± 1.71	0.33 ± 0.76	1.00 ± 1.72	6.92 ± 3.83

of bloom available. Additionally, because of the mobility of insects, particularily bees, it is to be expected that abundances at any given place and point in time should vary.

A common feature of the insect abundance data throughout is the great variability. As a result, when considered separately, analysis of bee and non-bee pollinator abundance patterns were largely inconclusive. However, the abundance patterns of all pollinating insects combined displayed definite trends. After first spray application abundances dropped noticeably within both treatment and control areas. The effects of spraying at this time however were masked by a large drop in air temperature which could have contributed to decreased insect abundances. However, while abundances subsequent to second spray application were significantly lower within the treatment area they were higher in the control transects when compared with pre-spraying abundances.

Qualitative observations made by field workers revealed that subsequent to spraying both bee and non-bee pollinators exhibited aberrant behaviour which ranged from general disorientation to immobilization. Such behaviour was observed only within spray areas.

5.2 Fruit Set Study

Results of the fruit set monitoring study revealed no differences in number of buds, flowers or fruit between control and treatment areas at the three major time periods. This was not surprising for several reasons. First, one must be able to distinguish between genetic and environmental (e.g. spray) influences on fruiting success (D. Larson, pers. comm.). The timing for Ranunculus spp. setting their seed is not known for Newfoundland although it is probable that bees would be attracted rapidly to these plants at time of flowering (B. Jackson, pers. comm.). Given that plants were already fruitin

prior to first spray application numerous plants may have already been pollinated in the treatment and control block, obscuring potential spray effects on fruiting success.

6.0 RECOMMENDATIONS

Results of this monitoring study and previous studies have revealed that monitoring methodologies currently employed are unsatisfactory in terms of producing standardized results. The problem arises from attempting to devise standardized field monitoring techniques. In contrast, laboratory tests are easily standardized.

Major problems in the field include lack of control over actively moving pollinators such as bees, lack of knowledge on fruiting periods of many Newfoundland plants and the interaction between pollinating insects and the various flowering plant species. A problem which also arises in short term studies is the potential coinciding of adverse weather (e.g. lowered temperature) at the time of spray application. Such a compounding factor makes it difficult to distinguish between environmental and spray effects. For instance, in this study, a temperature drop of ca. 14°C was recorded at the time of first spray application.

Overall, it is recommended that the current monitoring methodology employed in fenitrothion spray/pollinator activity studies be re-evaluated for improvement or total revision. This would allow standardized methodology to be incorporated into the terms of reference.

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FENITROTHION RESIDUE ANALYSIS IN BALSAM FIR FOLIAGE SAMPLES COLLECTED DURING THE 1985 AND 1986 HEMLOCK LOOPER FOREST SPRAY PROGRAM

Prepared by

Fudge, Lane and Associates Limited

under contract to

the Minister of Forest Resources and Lands

for

the Minister of Environment

1987

The attached study was submitted to the Minister of Environment by the Minister of Forest Resources and Lands as required under the Pesticides Operator License issued by the Minister of Environment for the 1986 Hemlock Looper Forest Protection Program.

The Minister of Environment brought together an expert committee to review the technical merit of this study and found it to be inconclusive.

FENITROTHION RESIDUE ANALYSIS IN BALSAM FIR FOLIAGE SAMPLES COLLECTED DURING THE 1985 AND 1986 HEMLOCK LOOPER FOREST SPRAY PROGRAM

PREPARED FOR:

DEPARTMENT OF FOREST RESOURCES AND LANDS
GOVERNMENT OF NEWFOUNDLAND AND LABRADOR
CORNER BROOK, NEWFOUNDLAND

SUBMITTED BY:

FUDGE, LANE & ASSOCIATES LIMITED
ST. JOHN'S, NEWFOUNDLAND

JANUARY 1987

F-108/109

EXECUTIVE SUMMARY

Objectives: To determine the vertical and horizontal distibution of aerially applied fenitrothion by analysis of forest vegetation samples and to relate this pattern of distribution to the results of concurrent impact studies on forest songbirds and pollinator insects.

Methods: Foliage samples from balsam fir were taken at various heights in the forest both along and perpendicular to the aircraft flight path and were assayed for fenitrothion concentrations. Control samples were taken for comparison.

Results: Upper canopy, mid-canopy and ground level foliage samples were combined and the group sample mean concentrations ranged from 0.06 µg/g to 8.84 µg/g. Individual residue concentrations in foliage samples ranged from <0.005 µg/g (i.e. no detectable residue) to 17.5 µg/g with a mean value of 1.22 µg/g.

Fenitrothion concentration decreased from upper canopy to ground level and from mid-canopy to ground level, although this general trend was not shown in all sample profiles. Analysis showed that upper and mid-canopy foliage had similar residue concentrations.

Results from the experiment designed to compare residue deposits in foliage along flight lines and across flight lines were inconclusive. Small sample sizes and large variation in the sample residue concentrations precluded rigid statistical analysis of the experimental results.

Conclusions: Fenitrothion applied aerially appears to concentrate at the upper canopy and mid-canopy level as would be expected. Definite conclusions regarding the effect of forest spray destination on forest songbirds and insect pollinators cannot be made and would require a more rigid experimental design to do so.

Recommendations: A review of sampling methods and/or experimental design are called for if all the objectives of the NDOE are expected to be achieved.

DFRL and NDOE should consider the future and usefulness of this residue study if significant changes in study objectives and/or experimental design are not considered.

STUDY TEAM

The study team consisted of the following personnel:

Stephen Fudge (M.Sc.), Biologist - Project Manager

David Lemon (B.Sc., B.Ed.), Biologist - Project Co-ordinator

Mr. Lemon was the field co-ordinator and prepared the final report.

Sandra Meron (B.Sc.), Biologist - Field Supervisor

Ms. Meron supervised the field collection of residue samples. She has an extensive background in work experience and education in invertebratology, entomology and botany.

Valarie Anstey (B.Sc.), Biologist and Barry Hicks (B.Sc. Pending), Biologist - Field Crew

Ms. Anstey and Mr. Hicks are biologists with academic and practical training in entomology and botany.

Mr. John L. Wells (B.Sc.), Biologist

Mr. Wells assisted in this report preparation.

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1.0 INTRODUCTION

The Newfoundland and Labrador Department of Forest Resources and Lands (DFRL) was requested by the Newfoundland and Labrador Department of Environment (NDOE) to conduct a residue monitoring study during the 1986 hemlock looper forest spray program. The insecticide used was fenitrothion. A balsam fir (Abies balsamea) foliage sampling study was designed by NDOE to determine the destination and concentration of fenitrothion residue vertically in vegetation exposed to spray. The NDOE also provided experimental design methods to assess the destination of fenitrothion residue along and across flight lines. The foliage residue sampling study was conducted along with studies to monitor the effects of fenitrothion on forest songbirds and pollinator insects.

1.1 Objectives

The objective of this study was to quantify the destination of aerially applied fenitrothion through foliage residue sampling and analysis. Results of the residue analysis were to be applied qualitatively to the results of the songbird and pollinating insect studies conducted simultaneously.

Fudge, Lane and Associates Limited on behalf of DFRL used foliage sampling methodology provided by NDOE for this study.

1.2 Study Area

The study area was located about 20 km south of Millertown, Newfoundland, within the Central Newfoundland Ecoregion (Damman 1983). The area is characterized by balsam fir (Abies balsamea) and black spruce (Picea mariana) forests. Stands of trembling aspen (Populus tremuloides) and white birch (Betula papyrifera) are common in the study area. Scrub areas of aspen and alder

(Alnus rugosa) and regenerating cut-over areas also occur in the study area. Dwarf shrub heath dominated by Kalmia angustifolia and small areas of Sphagnum spp. bog can occur locally.

Much of the study area was cut over in the mid to late 1960's. The regenerated areas are composed of immature (0-20 years) black spruce and balsam fir under 3.5 m in height, some mature black spruce (41-60 and 61-80 years) 9.6-12.5 m high and some areas which were not sufficiently restocked (Forest Inventory Maps DFRL). The location of the study area is shown in Figure 1.1.

1.3 Methodology

1.3.1 1985 Program

The results of the 1985 fenitrothion residue analysis were provided for this study by DFRL. They were not collected using the same sampling procedure described for 1986. However, the amount of foliage collected, preservation techniques and chemical analysis are similar for both 1985 and 1986.

1.3.2 1986 Program

The 1986 fenitrothion residue sampling procedure was provided by the NDOE (Figure 1.2). Foliage samples from balsam fir were collected from the representative vegetation in the spray block, near the pollinating insect monitoring study area and near the forest songbird monitoring study area (Figure 1.3).

All foliage samples were collected within 4 h of the spray application.

Figure 1.1. Location of study area.

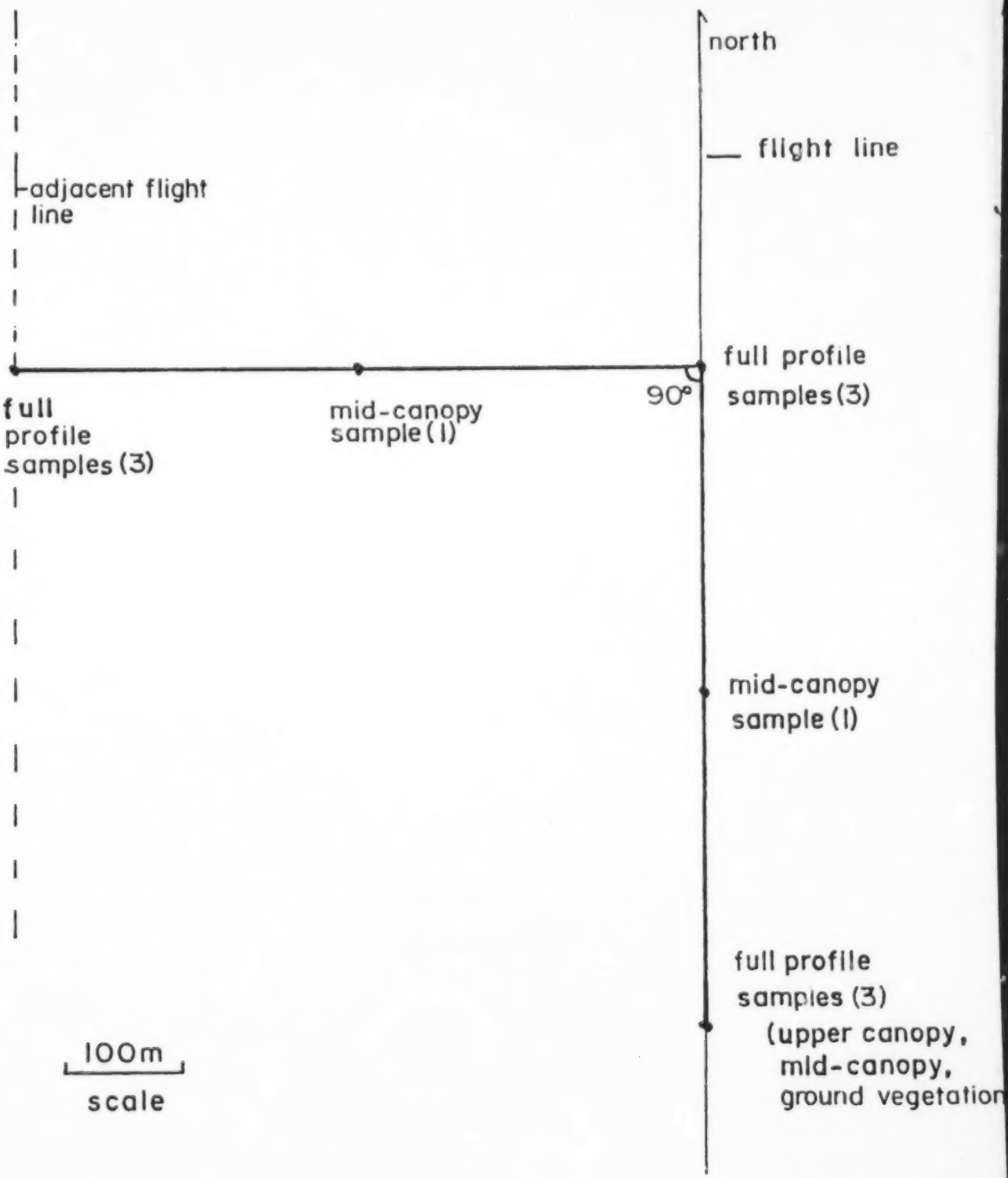

Figure 1.2. Sampling methods used to compare residue concentrations in balsam fir foliage along and across flight lines.

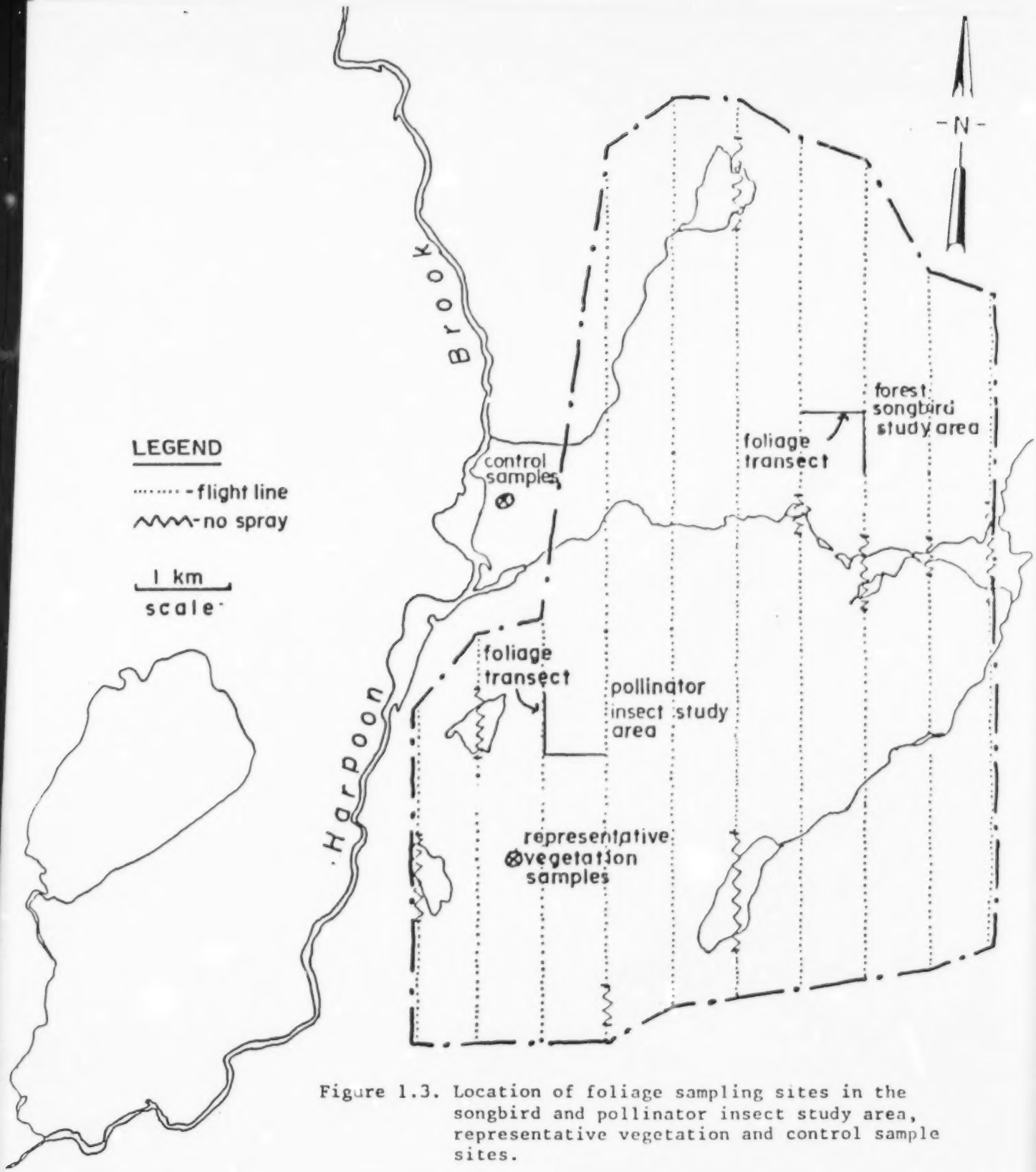

Figure 1.3. Location of foliage sampling sites in the songbird and pollinator insect study area, representative vegetation and control sample sites.

1.3.3 Destination of Residue (1986 Program)

1.3.3.1 Representative Vegetation - Mature

Foliage samples were collected from representative mature vegetation in the spray block area. Three foliage samples were collected at each of 10 locations (i.e. 10 mature trees). Tree locations were selected at random. At each sampling location a 100 g foliage sample was collected from the upper canopy level (8-10 m), the mid-canopy level (3-6 m) and the ground level (0-1 m) foliage.

Four of the 10 sites were sampled after the first spray application of fenitrothion, four sites were sampled after the second spray application of fenitrothion and 2 sites were sampled as controls (outside the spray block). Thirty samples were collected in all. Sampling location is shown in Figure 1.3.

1.3.3.2 Representative Vegetation - Regeneration

Foliage samples were collected at 10 sample sites in areas of regenerating vegetation, i.e. balsam fir. Samples were collected from regenerating vegetation (1-4 m in height), only 1 sample was collected at each site (tree).

Four sites were sampled after the first spray application and 4 sites were sampled after the second spray application. Two control samples were collected from regenerating vegetation areas outside the spray block. Ten samples were collected in all.

1.3.3.3 Representative Vegetation - Scrub

Foliage samples were collected at 10 sample sites in areas of scrub vegetation (balsam fir). Samples were collected from ground vegetation (0-1 m in height). Four sites were sampled from scrub vegetation after the first spray application and 4 sites were sampled after the second spray application. Two control samples were collected from scrub vegetation areas outside the spray block. Ten samples were collected in all.

1.3.3.4 Sampling Along Flight Line

Foliage samples were collected parallel to and directly under one of the flight lines used for spraying. At a point along the flight line in the area of the pollinator insect monitoring study a sampling transect was initiated. At the beginning of the transect an upper canopy, mid-canopy and ground level foliage sample was collected. Further along the transect at approximately 300 m a mid-canopy sample was collected, then at the end of the transect approximately 600 m from the beginning a full profile (canopy, mid-canopy and ground vegetation) sample was collected again. This procedure was followed after the first and second spray applications.

This sampling procedure was conducted in the songbird monitoring study area after the first and second spray applications as well. Eleven samples were collected after each spray application in each study area.

1.3.3.5 Sampling Across Flight Line

Foliage samples were collected perpendicular to spray flight lines on transects beginning at the end of the transects described in Section 1.3.3.4. Beginning with the full profile (upper canopy, mid-canopy and ground vegetation) samples collected at the end of the transects described in 1.3.3.4 a transect perpendicular to the flight line was walked. At approximately 300 m along this perpendicular transect a mid-canopy sample was collected. At a point approximately 600 m from the end of the transect parallel to the flight line a full profile (upper canopy, mid-canopy and ground vegetation) sample was collected. This procedure was conducted after the first and second spray application.

Eleven samples were collected (including 3 full profile samples) at end of the transect along flight line) after each spray application. This procedure was performed in both the pollinator insect and songbird study areas.

Full profile samples were collected in a control (non-sprayed) area after each spray application for a total of 6 samples.

1.3.3.6 Codroy Pond Overspray

On 19 July 1986 a total of 23 residue samples were taken from spray block 101 (Figure 1.1) along a flight line which had been oversprayed on the previous morning. The overspray incident

was reported to the field crew by DFRL. The sampling methods were the same as those used in the study spray block #107.

1.3.3.7 Residue Sampling

The residue sampling scheme to compare concentrations amongst samples collected along and across flight lines is shown in Figure 1.2. The approximate locations of foliage collection sites are shown in Figure 1.3.

Representative vegetation samples were collected at approximately 48° 39' 37"N, 56° 31' 32"W.

Control samples were collected at approximately 48° 41' 27"N, 56° 31' 32"W.

Residue samples on transects in the pollinating insect monitoring study area were collected at approximately:

0 m N	48° 39' 54"N, 56° 31' 05"W
300 m N	48° 39' 48"N, 56° 31' 14"W
600 m N	48° 39' 34"N, 56° 31' 19"W
300 m E	48° 39' 31"N, 56° 31' 02"W
600 m E	48° 39' 29"N, 56° 30' 51"W

Residue samples on transects in the forest songbird monitoring study area were collected at approximately:

0 m N	48° 41' 53"N, 56° 28' 32"W
300 m N	48° 41' 42"N, 56° 28' 37"W
600 m N	48° 41' 32"N, 56° 28' 37"W
300 m W	48° 41' 52"N, 56° 28' 46"W
600 m W	48° 41' 55"N, 56° 29' 00"W

All sampling locations can be found on the Lake Ambrose map sheet (1:50,000 scale 12A/10) produced by the Surveys and Mapping Branch, Department of Energy, Mines and Resources. A portion of this map sheet is reproduced in Figure 1.3.

A portion (10 swaths) of block #107 was sprayed to enable the monitoring study to proceed. Spraying was conducted using one DC-4 aircraft equipped with boom and nozzle spray apparatus.

Technical details of spray application and mixture are provided in Table 1.1.

2.0 RESULTS

The results of the fenitrothion residue analysis from foliage samples are shown in Table 2.1.

The mean foliage residue sample, excluding the samples collected in control areas and in the Codroy Pond overspray incident, is 1.22 µg/g (ppm).

Data from Table 2.1 was grouped to show the vertical distribution of residue concentrations in the forest vegetation. Figure 2.1 shows the vertical distribution of residue in balsam fir foliage when the 1985 and 1986 results are grouped.

Figure 2.2 shows the vertical distribution of residue in balsam fir foliage when data available from full profile samples are grouped.

The Codroy Pond overspray incident foliage samples were not included in the vertical distribution of residue analysis because foliage samples were collected more than 4 h after the spray application and greater than operational spray concentrations of fenitrothion were used.

Spray Application

Aircraft:	DC-4
Aircraft height:	61 m
Aircraft speed:	305 km/h
Spray apparatus:	Boom and nozzle with #8010 spray tip

Spray Mixture

Insecticide:	fenitrothion (Folithion^{R})
Mixture by Volume:	11% fenitrothion
	40% Cyclosol 63
	49% ID 585 (Insecticide Diluent)

Application Rate

210 g active ingredient per 1.5 L per ha in 2 applications.

Application Date

1st. spray 27 July 1986 (evening)
2nd. spray-partial 2 August 1986 (evening)
-partial 3 August 1986 (morning)

TABLE 1.1: Technical details of spray application and formulation for 1986 hemlock looper control program.

TABLE 2.1: Fenitrothion residues detected in balsam fir foliage samples collected within four hours after spraying, 1985 and 1986, in µg/g (ppm) (fenitrothion applied at 210 gAI/ha).

	Spray Application/Location	Canopy $\bar{X}$ (S.D.) <n>	Mid-Canopy $\bar{X}$ (S.D.) <n>	Ground $\bar{X}$ (S.D.) <n>	Control[2] $\bar{X}$ (S.D.) <n>
1985					
1	DFRL Spray Block 201	2.37 (1.39) <6>	ns*	0.28 (0.28) <6>	No residue detected.
2	DFRL Spray Block 305	1.14 (0.33) <6>	ns	0.90 (0.58) <6>	No residue detected
3	DFRL Spray Block 207 (1st. Application)	1.76 (0.45) <4>	ns	1.63 (0.77) <4>	No residue detected
4	DFRL Spray Block 207 (2nd. Application)	3.55 (1.15) <3>	ns	1.03 (0.14) <3>	No residue detected
5[1]	DFRL Spray Block 312	0.82 (0.22) <3>	ns	1.90 (1.97) <3>	No residue detected
1986					
6	DFRL Spray Block 107 Mature Forest (1st Application)	0.61 (0.27) <4>	0.43 (0.16) <4>	0.32 (0.16) <4>	0.12 (0.12) <6>

* No Samples
S.D. = Standard Deviation
n = Sample Size
[1] Block 312 sprayed with 280 gAI/ha fenitrothion
[2] Control samples were grouped due to overall low fenitrothion values

TABLE 2.1 (Cont'd)
Page 2

Spray Application/Location		Canopy $\bar{X}$ (S.D.) <n>	Mid-Canopy $\bar{X}$ (S.D.) <n>	Ground $\bar{X}$ (S.D.) <n>	Control[2] $\bar{X}$ (S.D.) <n>
1986					
7	DFRL Spray Block 107 Mature Forest (2nd Application)	0.15 (0.09) <4>	0.10 (0.07) <4>	0.07 (0.06) <4>	0.12 (0.12) <6>
8	DFRL Spray Block 107 Regeneration Forest (1st Application)	ns	0.63 (0.60) <4>	ns	0.01 (0.01) <4>
9	DFRL Spray Block 107 Regenration Forest (2nd Application)	ns	0.13 (0.08) <4>	ns	"
10	DFRL Spray Block 107 Scrub Vegetation (1st Application)	ns	ns	0.37 (0.08) <4>	"
11	DFRL Spray Block 107 Scrub Vegetation (2nd Application)	ns	ns	0.07 (0.03) <4>	"

TABLE 2.1 (Cont'd)
Page 3

Spray Application/Location		Canopy $\bar{X}$ (S.D.) <n>	Mid-Canopy $\bar{X}$ (S.D.) <n>	Ground $\bar{X}$ (S.D.) <n>	Control[2] $\bar{X}$ (S.D.) <n>
1986 - Pollinator Study Area Spray Block 107					
12	North transect directly under and paralleling swath (1st Application)	0.69 (0.75) <2>	0.27 (0.41) <3>	0.06 (0.08) <2>	0.01 (0.01) <3>
13	East transect perpendicular to north transect (1st Application)	1.45 (1.82) <2>	0.06 (0.03) <3>	0.96 (1.36) <2>	"
14	North transect (2nd Application)	2.97 (4.04) <2>	2.03 (1.57) <3>	0.27 (1.57) <2>	0.06 (0.02) <3>
15	East transect (2nd Application)	3.01 (3.99) <2>	1.21 (1.83) <3>	0.33 (0.12) <2>	"
1986 - Songbird Study Area Spray Block 107					
16	North transect directly under and paralleling swath (1st Application)	8.84 (12.25) <2>	3.56 (2.20) <3>	0.07 (0.14) <2>	0.01 (0.01) <3>

TABLE 2.1 (Cont'd)
Page 4

Spray Application/Location		Canopy $\bar{X}$ (S.D.) <n>	Mid-Canopy $\bar{X}$ (S.D.) <n>	Ground $\bar{X}$ (S.D.) <n>	Control[2] $\bar{X}$ (S.D.) <n>
1986- Songbird Study Area (Cont'd) Spray Block 107					
17	West transect perpendicular to north transect (1st Application)	0.09 (0.12) <2>	5.63 (8.38) <3>	0.11 (0.01) <2>	0.01 (0.01) <3>
18	North transect (2nd Application)	0.61 (0.27) <2>	5.50 (6.40) <3>	0.09 (0.08) <2>	0.06 (0.02) <3>
19	West transect (2nd Application)	0.54 (0.17) <2>	3.69 (3.40) <3>	0.20 (0.08) <2>	"
1986 - DFRL Spray Block 101					
20	Codroy Pond - overspray incident	0.38 (0.33) <7>	0.28 (0.30) <7>	0.11 (0.08) <9>	ns

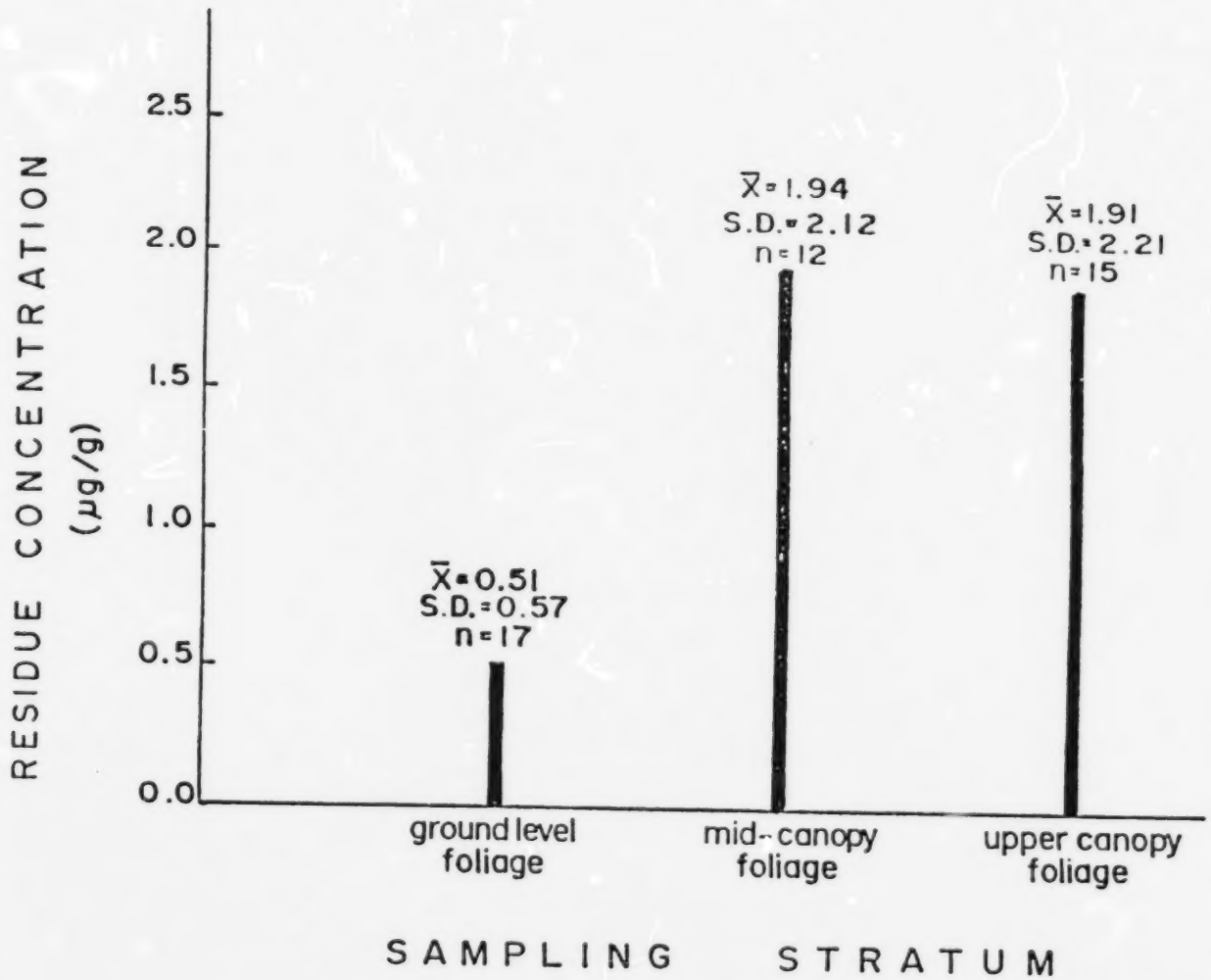

Figure 2.1. Mean ($\bar{X}$) fenitrothion residue concentration (µg/g) observed in upper canopy, mid canopy and ground foliage samples, 1985 and 1986 data combined. (S.D. = standard deviation, n = sample size).

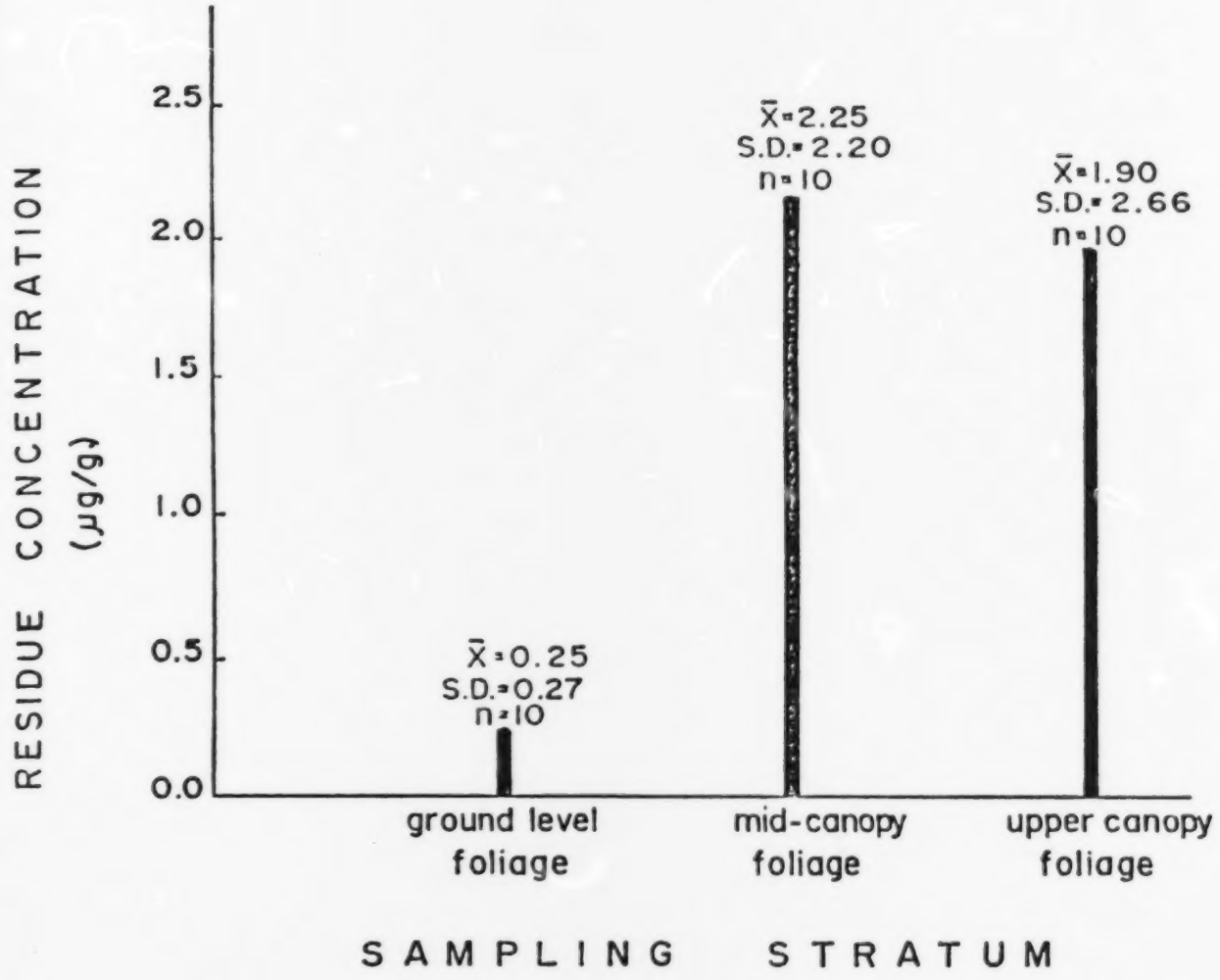

Figure 2.2. Mean ($\bar{X}$) fenitrothion residue concentration (µg/g) observed in upper canopy, mid canopy and ground foliage samples using data when all three strata samples were collected, 1986 data only. (S.D. = standard deviation, n = sample size).

3.0 DISCUSSION

The foliage sampling procedure used in this study was intended to indicate the range in variability in fenitrothion residue concentrations in foliage samples. The results in Table 2.1 show considerable variability within and between sampling stratum (i.e. upper canopy, mid-canopy and ground vegetation). In any one spray application there are large standard deviations in very small sample sizes and comparisons of the same stratum between spray applications also show large variations.

The large variations in the sample groups and the small sample sizes preclude rigorous statistical analysis of the data.

The general trend in Table 2.1 shows higher residue concentrations in foliage collected in the upper canopy followed by foliage collected at the mid-canopy then the ground foliage samples. This result is expected in spray applications since the higher portions of the tree would intercept the spray before the lower portions of the tree. However this pattern is not observed in all cases (see rows 5, 17, 18 and 19 in Table 2.1).

A further analysis of the sample group means in Table 2.1 shows that the upper canopy foliage samples and the mid-canopy foliage samples contain similar concentrations of residue (Figures 2.1 and 2.2). The residue concentrations drop off considerably in ground foliage samples. This trend is more likely explained by the shape of the fir trees. The common conical shape would allow mid-canopy and upper canopy areas unrestricted deposition of spray residues and considerably reduce the amount of spray reaching the ground level foliage.

The control samples in 1986 were collected outside the spray block and some were contaminated with small amounts of fenitrothion residue.

The presence of small amounts (<0.01 µg/g <ppm>) of fenitrothion residue in control samples collected in unsprayed areas adjacent to spray blocks identifies the problem of non-target areas being subjected to fenitrothion spray through drift. However, small amounts of fenitrothion detected in foliage collected outside the study block are not to be interpreted as an operational failure to control spray dispersion. Varty (1980) concludes that concentrations and persistences of drifted fenitrothion represent the sensitivity of analytical techniques more than a failure to control spray dispersion.

The large variation in residue concentrations observed in this study was also observed in balsam fir foliage collected between 1979 and 1982 in Quebec. Morin et al. (1986) observed variations so large that they used median values rather than arithmetic means to describe their results. They estimated that the sample group median value was more representative of the concentrations generally expected in the environment after spraying (Morin et al. 1986). However, creating sample sizes large enough to reduce the variation observed in this study enabling the use of median values rather than mean values would preclude determining the precise destination of fenitrothion in the forest strata, and along and across application flight lines.

The results of the study to compare the residue concentrations in foliage deposited along the flight line and perpendicular to the flight line (Figure 1.2) are inconclusive. The foliage samples collected along the perpendicular transect resulted in the two full profile samples lying directly under adjacent application flight lines. Therefore, only one mid-canopy sample between flight lines remains to assess the variation in residue deposition perpendicular to the application flight lines. One sample is inadequate for meaningful analysis especially in view of the large variation shown in the results.

The sampling methodology provided by NDOE to compare the residue concentrations in foliage along and across flight lines is considered inadequate to achieve this objective.

The objective to use the fenitrothion residue analysis to provide meaningful data to better interpret the results of the concurrent forest songbird and pollinator insect monitoring studies was not achieved due to the experimental design of the residue study.

Until a more rigid experimental design can be employed to account for the large variations observed in residue results within and between sampling strata, attempting to relate the results of this study to the monitoring studies of songbirds and insects, which are continually moving throughout the study area, is considered ambitious and would amount to conjecture.

4.0 CONCLUSIONS

The results of this study, incorporating the 1985 and 1986 foliage residue results, indicate that foliage samples collected from the mid-canopy and upper canopy regions of the forest usually had higher fenitrothion residue concentrations than foliage samples collected at ground level.

No conclusions can be made from the foliage sampling experiment aimed at comparing the differences in spray deposition along and across application flight lines due to methodology problems.

The experimental design provided for this study and the amount of variation encountered in the results do not permit the application of foliage residue results to either the songbird or pollinator insect monitoring studies.

The small number of foliage samples at any particular stratum and the large variation in results within the sample groups combined with the songbirds and insects moving throughout the forest and not knowing their location when the spray was applied; and how much and when the organisms ingested the residue makes attempts to collaborate the results of these studies impossible.

5.0 RECOMMENDATIONS

The methodology used in this study is useful for determining trends and variations in the destination and concentration of fenitrothion in foliage during forest spray applications.

However if this study is going to provide meaningful results to be used in collaboration with songbird and pollinator insect monitoring studies a review of the experimental design is recommended.

The methodology used in the 1986 study provided very small sample sizes. The large variations within these small sample groups do not provide useful results for statistical analysis.

The DFRL and NDOE should consider the future and usefulness of this study if significant study changes in methodology and/or objectives are not considered.

6.0 LITERATURE CITED

Morin, R., Gaboury, G. and G. Mamarbachi. 1980. Fenitrothion and aminocarb residues in water and balsam fir foliage following spruce budworm spraying programs in Quebec, 1979 to 1982. Bull. Environ. Contam. Toxicol. 36:622-628.

Varty, I.W. 1980. Summary overview of environmental surveillance in 1978 and 1979. In: Environmental surveillance in New Brunswick, 1978-79 effects of spray operations for forest protection against spruce budworm. EMOFICO report. Department of Forest Resources, University of New Brunswick, Fredericton, NB.

APPENDIX I

DETERMINATION OF FENITROTHION
WATER ANALYSIS FACILITY, FALL 1986

Introduction

This method was used for the analysis of foliage and moss for fenitrothion during the fall of 1986 at the Water Analysis Facility, Memorial University, St. John's, Newfoundland.

The analyses were done by Art Cook and Robert Redmond and were completed November 21, 1986. Analysis methodology was provided for this report on January 13, 1987.

This method is based on Reynolds, L.M., Determination of Fenitrothion and Matacil Residues in Newfoundland Samples, ORF, 1977.

Materials and Reagents

Fenitrothion analytical standard
Solvents - all distilled in glass
Florisil - 60/100 mesh, heated at 600°C overnight, then deactivated with 2% water
Florisil columns - 2.5 x 30 cm, glass frit with ground glass stopcock
Buchner filter funnels
Waring commercial blender
Porcelain mortar and pestle
Buchi rotary evaporator
Assorted lab glassware, solvent rinsed

APPENDIX 1 (Cont'd)

Gas Chromatography Equipment and Operating Conditions

Varian 3700 GC
Column - 30 m DB-5 capillary
Temperature program - initial 85°C for 2 min., then 15 C/min. to 230°C final, and 20 min. at final temperature
Detector - electron capture
Carrier glass - column nitrogen 2.7 mL/min. (18 psi), hydrogen 5.8 mL/min. (27 psi), makeup 35 mL/min., air 178 mL/min., purge 12 mL/min.
Integrator - Hewlett-Packard 3380A

Method

1. More than ten grams of sample was placed in a mortar and frozen with liquid nitrogen. The frozen sample was ground with a pestle and allowed to thaw. Ten (+/- 0.01) grams of sample was placed in a blender with 100 mL ethyl acetate and blended for 30 s at full power.

2. The blended sample was poured into a Buchner funnel and the blender rinsed with two portions of ethyl acetate to make up 250 mL total volumn. The solvent was pulled through with gentle suction.

3. The solvent was transferred to a round-bottomed flask, taken to dryness and taken up in 5 mL of 35% methylene chloride in hexane.

4. Columns were made up by wet packing 12 cm florisil in hexane into the 2.5 cm glass columns, and adding 1 cm sodium sulfate to the top.

5. The sample was transferred to the top of the column and eluted with 250 mL 35% methylene chloride in hexane.

APPENDIX I (Cont'd)

Method (Cont'd)

6. The eluate was concentrated to almost-dryness on a rotary evaporator and taken up in 2.0 mL ethyl acetate for GC analysis.

7. Injections of 0.8 µL were made in duplicate, with daily standards. Determinations were made by comparison of electronically integrated peak areas.